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ORIGINAL ARTICLES

A RAPID QUANTITATIVE METHOD FOR THE COMPARISON OF DIURETIC AGENTS IN BED-PATIENTS WITH CONGESTIVE FAILURE*

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In the present report we describe a method for the quantitative comparison of diuretic agents with bed-patients in congestive failure. The data emerge out of the effects of a standard regimen of therapy. The "test-agent" is assayed against a "standard" in the same patients by paired comparisons of 24-hour weight loss responses to a series of "ceiling" doses of each given concurrently. Diuretic efficacy is computed with confidence limits from the ratios of the measured responses in a group of patients, and from an analysis based on signs with the sign test for the reliability of the answer.

The possibility for such a method was suggested by the experience that

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24 hours after an effective dose of an organic mercurial diuretic a patient with congestive failure shows a loss of body weight, that in terms of 24-hour weight losses satisfactory dose-response relationships can be demonstrated (Clarke *et al.*[2]), and by the observations made in the course of a study of a simple regimen for the treatment of congestive failure (Gold *et al.*[6]), namely, that a dose of the mercurial given daily to the patient on a constant diet often provides a series of 24-hour weight losses, which makes a fairly smooth curve.

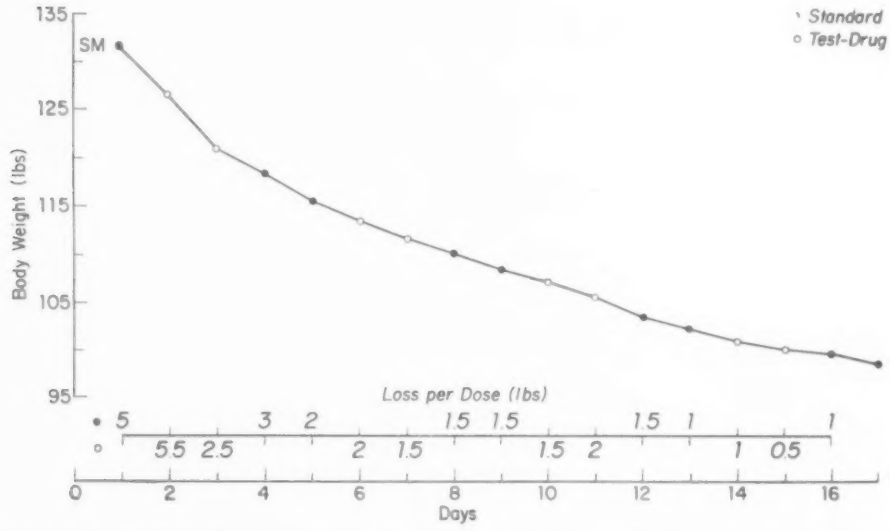

Fig. 1.—Comparison of meralluride, 2 ml. as the "standard" with 2 ml. as also the "test-drug" in the ABBA pattern. Patient, male, 44, hypertensive and arteriosclerotic heart disease, sinus rhythm, far advanced congestive failure. He had been digitalized and upon admission was placed on a daily 0.2 mg. dose of digitoxin, a daily diet of milk and water, 1.5 liters each, and a daily intramuscular injection of 2 ml. meralluride. He lost nearly one-fourth of his body weight with clearing of the symptoms and signs of failure. Points on curve refer to the drug and numbers below, corresponding 24-hour weight changes.

Figure 1 illustrates the general character of the curve. The case is that of a patient referred to one of us (H.G.) about 14 years ago for treatment of a far advanced stage of congestive failure. In the hospital the essential treatment was a maintenance dose of 0.2 mg. digitoxin, a diet of 2 to 3 liters of milk and water, and a 2 ml. intramuscular dose of meralluride daily. The weight began to fall promptly and continued steadily until about 33 lbs. of edema fluid were lost, or about one-fourth of his body weight, with the clearing of the symptoms of failure. It may be seen in this case that with minor variations, the ratios in successive pairs of responses agreed with the expected 100. It is pertinent here to call special attention to the fact that this curve is constructed from the data in Fig. 1 of a paper we published 12 years previously (Gold *et al.*[6]), showing the responses in a routine course of treatment.

Figure 2 is an example of the same kind of an experiment in which the

2 ml. dose of meralluride alternated with 1 ml. After four doses (2 doses of each), the patient lost 22.5 lbs., 14 of these with the 2 ml. doses, and 8.5 with the 1 ml. doses, yielding an efficacy ratio of 61.6.

Such observations indicated meaningful quantitative comparisons and the precision of the answers, and the competence of the method to yield values in agreement with expected ones in the case of materials with known relationships. A preliminary report of the method was presented at the Spring Meeting of the Pharmacological Society (Gold *et al.*[7]).

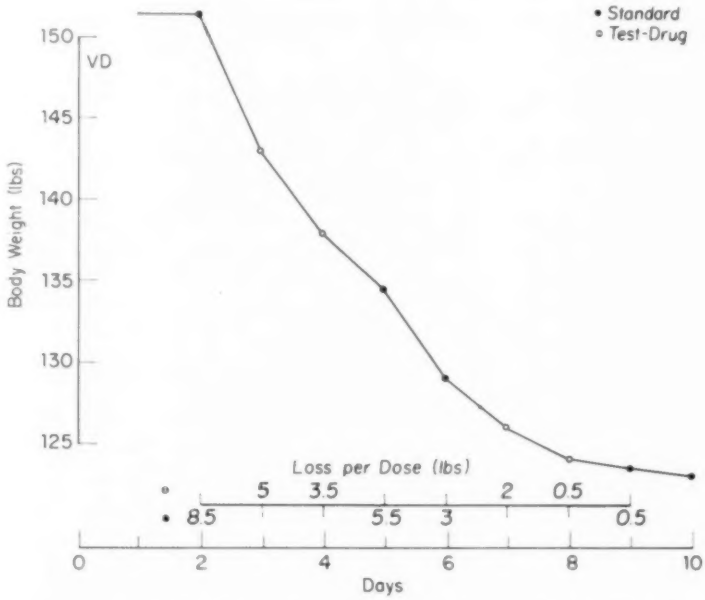

Fig. 2.—Comparison of meralluride, 2 ml. with 1 ml. Patient, male, age 73, hypertensive and arteriosclerotic heart disease, syphilis, atrial fibrillation, far advanced congestive failure. Digitalization was maintained with 0.1 mg. digitoxin daily. He received a 2 gm. salt restricted diet with free water intake and starting on the second day, a daily intramuscular dose of meralluride, 2 ml. alternating with 1 ml. in the pattern ABBA. He lost 32.5 lbs. with clearing of symptoms and signs of failure. Points on curve refer to the drug and numbers below, corresponding 24-hour weight changes.

suggested that the ratio of the measured responses to a succession of doses alternating between two agents, a standard and a test-drug, might provide a useful expression of the relative efficacy of two diuretics. This investigation was planned to determine the conditions necessary to apply such responses in a practical plan for assay, to explore the possible sources of error,

CEILING DOSES. In the conventional method of bio-assay, two compounds are compared with respect to the amounts of each which produce the same effect and the ratio of the doses is an expression of their relative "potency." For such an expression, the ratios of the responses to similar doses of the two agents may prove misleading because of the S-shaped character

of the dose-response relationship. However, if one uses "ceiling doses" of the two agents under comparison, that is, doses which evoke the greatest effect possible with each agent no matter how large the dose, the ratio of these responses which may be termed "ceiling effects," provides a valid means of comparison. Since the results do not reveal how much of each is necessary to produce the same effect, but which of the two is the more effective agent, or which possesses the greater therapeutic potential for a patient in congestive failure, to avoid confusion, the term relative "potency" is here replaced by relative "efficacy." Since the time for the response is the same for both agents, namely, 24 hours, the corresponding weight may be compared for an expression of the difference in the length of time it might take to clear the edema if each were used alone.

Meralluride (Mercuhydrin) by intramuscular injection was employed as the standard of comparison in our previous method of bio-assay (Greiner *et al.*[8]) and is continued in the new method. It is the general experience that a 2-ml. dose usually elicits the full or major part of the effect of this agent, and in the dose-response curve mentioned above, based on 971 doses in 73 ambulant patients with congestive failure, this appeared to be the approximate "ceiling dose" (Fig. 3). In the case of a new compound for assay, the "ceiling dose" needs to be determined by preliminary tests. This need not be established with any great precision since a dose considerably above the "ceiling" should not affect the results of the assay. For a new compound, especially a drug given by oral administration, it may not be feasible, because of toxic effects, to establish what may be termed the "pharmacologic ceiling dose," that is, the dose representing the maximal diuretic potentiality of the agent. For practical purposes in preliminary testing this is not necessary, since here the need is for a "therapeutic ceiling dose," namely, the dose which yields the maximum

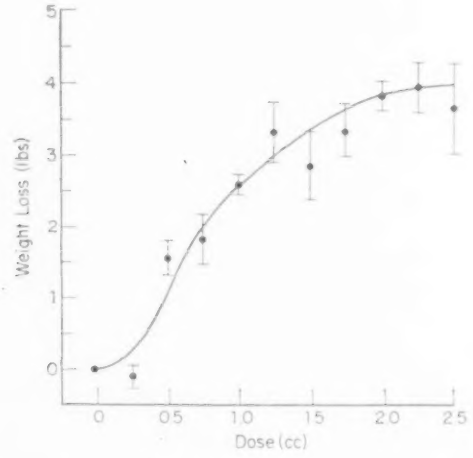

Fig. 3.—Dose-response curve of meralluride based on 971 intravenous injections in 73 ambulant patients in moderate congestive failure (see ref. 1). Brackets show standard error of the mean for each dose level.

diuretic response with an acceptable incidence of toxicity. The incidence of toxicity then becomes the deciding limiting factor and information about this renders less important precise values for the diuretic effects. These matters simplify the preliminary problem of establishing the "ceiling dose" necessary for the bio-assay.

TEST-PATIENTS. Most patients with congestive failure who show a 24-hour weight loss with the mercurial are satisfactory subjects for an assay. Those in moderately severe failure with an estimated 15 or more pounds of edema fluid supply the largest proportion of suitable data. Extreme grades of failure are sometimes unsatisfactory because they may fail to respond to even the most effective diuretic (intractable edema). The milder cases sometimes present these problems: (1) The base-regimen (bed-rest, salt-restriction digitalization) is apt to be so effective as to clear the edema rapidly by itself; (2) a potent diuretic may clear the edema in one dose; (3) patients with only a few pounds of available edema are often insensitive or erratic, and in them the last member of a pair may find insufficient fluid for the full action of the dose, an observation which emerged from an analysis of the results in 20 patients with 4 lbs. or less to lose.

PAIRED RESPONSES. From our present experience the indications are that the first four doses supplying 24-hour weight losses (2 pairs) provide adequate data for the paired comparisons in the majority of cases. The following restrictions should be observed:

(1) The data of the day of admission are better omitted because of the change in environment and diet, and because the weight change by the following morning before breakfast often represents less than a 24-hour period.

(2) In those receiving a digitalis material, it is well to take the data during the period of maintenance dosage.

(3) The responses for the paired comparisons should be taken from the declining portion of the weight curve during a succession of doses, not from the flat portion which usually represents the "dry-weight."

(4) No pair should be included without evidence that the condition of the patient is suitable to reveal the full action of the second member of the pair, a fact which can be ascertained from the continued decline of the weight curve with a few additional doses beyond the point of the assay pairs.

(5) In the event there appear complicating disorders or diseases such as periods of anorexia, nausea or vomiting, constipation, fever, infections, and others which manifestly affect body weight, the assay is interrupted until recovery takes place, or the patient is eliminated from the assay.

Procedure. The test-patient is placed at bed-rest and at once on an effective system of therapy for congestive failure. There is no so-called "control" period to "stabilize" the patient on rest and diet alone as is often done in clinical evaluations. The design of the assay has a self-contained "control" in the use of the two drugs concurrently with the sequence of reversing pairs and with patterns alternating from patient to patient.

During the period of assay the patient receives the required daily maintenance dose of a digitalis material. The initial digitalization is carried out before the assay.

The diet is salt-restricted, usually limited to approximately 2 gm. sodium chloride. We often employ a diet of milk and water, 1.5 L. of each. This is more constant than most of the hospital salt-restricted diets. Close supervision is maintained to insure that the glass of milk or water at hourly intervals is actually consumed. One cannot over-empha-

size the importance of close supervision to insure constancy in the matter of salt intake, total food and fluid consumption during the few days of the assay. In carrying out the routine order for the food and fluids, lapses are frequent all along the line, which may involve any or all of the persons concerned with the diet, namely, the dietitian, kitchen help, tray delivery personnel, the patient, the visiting family and friends. Irregularities with respect to diet are a major factor in the erratic patterns, usually erroneously assigned to erratic behavior of the patient in response to the diuretic.

The patient is weighed daily to the nearest 0.25 lb. on the same balance, checked for proper performance each time, carried out at the same time each day before breakfast under conditions which are constant with respect to manifest factors which may influence weight in this range such as clothing, bowel, bladder. This should be done by a person alert to the sources of error. In the present study the daily weighing was made by a physician collaborator. It would be superfluous to belabor these cautions in weighing or the importance of accuracy, if it were not for the fact that the weight changes constitute the crucial data and the casual manner in which a request for a daily weighing as commonly carried out usually provides values that are not sufficiently reliable for use in a bio-assay. Error in weighing is another major cause of erratic responses, also commonly ascribed to erratic behavior of the patient. Our experiences with the weighing of the patients by the general ward personnel checked by the professional person participating in the investigation have amply justified our concern with the details of the daily weighing.

Two diuretics are included in the therapeutic regimen. One is the test-agent and the other, 2 ml. intramuscular meralluride as the standard of comparison.

For the sequence in a series of doses, we have adopted the patterns ABBA and BAAB for alternate patients, A the "standard" and B the "test-drug," in order to eliminate a bias which might result if one of the agents always preceded the other. The bias may be quite considerable in individual patients receiving a long series of daily doses. For example, in one patient with about 60 lbs. of edema, who received a series of 20 daily oral doses of 2 gm. of a diuretic, the pattern ABBA applied to the 24-hour weight losses yielded the expected B/A ratio of 100, while the pattern ABAB yielded a B/A ratio of 84.8, hence a 15.2% bias in favor of the agent which always came first in the pair. It may be worth noting, however, that in similar tests applied to 20 patients of Table 1, the results showed the bias small and unimportant in situations resembling more closely those of the actual assay, namely, only a few doses in groups of patients.

The dose of a diuretic was given daily in some patients and every other day in others. The drug-free day provided an opportunity to obtain evidence on the question as to whether the 24- or 48-hour period of response to a dose is the more favorable for comparison of two drugs, also information on the role of the base-regimen in the responses assigned to a drug. The base-regimen (rest, salt-restriction, digitalization) may exert a diuretic effect and, were this equally distributed between the two drugs, there is the possibility of a biased ratio in favor of the weaker drug. In such a system a diuretic effect might be assigned even to a placebo. An examination of our data on the drug-free day has revealed no satisfactory means of adjustment to secure efficacy ratios for the drugs, free of the influence of the base-regimen. The latter factor is therefore included in the comparisons by the present method, as it is in most clinical evaluations of diuretics in patients in congestive failure. It is of interest to note that in a group of 24 patients who received 123 intramuscular doses of 2 ml. meralluride the first 24-hour period showed a weight loss with every dose, which averaged 2.6 lbs. S.E. $\pm$ 2, while the drug-free day which followed showed either no change, a further loss, or a gain, the net result a gain of 0.1 lb.

At present we use the 24-hour period for the measured response. We are not yet certain whether this or the 48-hour period will prove to provide the more stable values in the drug comparisons.

UNIFORMITY TEST. The competence of the method was challenged in two ways: (1) a known drug similarity, and (2) a known drug difference.

For the first challenge, the data were assembled from 20 of the patients with congestive failure who had undergone a course of therapy we described in the previous report[6]. Most of the data came from the routine treatment in that study which was made more than 10 years prior to the planning of the present method of assay. We mention

TABLE 1.—WEIGHT LOSS WITH 2 ML. INTRAMUSCULAR MERALLURIDE DAILY BY PATIENTS IN CONGESTIVE FAILURE ON A STANDARD REGIMEN OF FAILURE THERAPY.

Pt. No.	*Name*	*Sex*	*Age*	*Diagnosis*	*Pattern*	*Edema (lbs.)*	*Weight Losses (lbs.)*							
							First pair		*First 2 pair*		*First 3 pair*		*First 4 pair*	
							S	*T*	*S*	*T*	*S*	*T*	*S*	*T*
1	R.P.	M	57	As,Hyp,Cor Thr,EH,RSR	ABBA	8.25	3.5	3.25	—	—	—	—	—	—
2	W.G.	M	80	As,Hyp,EH,RSR	BAAB	5	2	2	—	—	—	—	—	—
3	R.A.	M	52	As,Hyp,EH,RSR	BAAB	8	3.5	3.5	—	—	—	—	—	—
4	J.B.	M	73	As,Hyp,Cor Thr,AI,EH,RSR	ABBA	11.5	5	3.25	—	—	—	—	—	—
5	L.M.	M	59	As,Hyp,EH,RSR	BAAB	13	4	3	—	—	—	—	—	—
6	I.L.	F	53	Rh,MS,MI,AI,EH,AF	ABBA	21	1.5	1.5	—	—	—	—	—	—
7	T.D.	M	42	Rh,MS,MI,EH,RSR	BAAB	30.5	8	11	—	—	—	—	—	—
8	E.M.	F	66	As,Hyp,EH,RSR	ABBA	13.5	2.75	3.75	5.25	5.25	—	—	—	—
9	A.M.	M	49	As,Hyp,EH,RSR	BAAB	22	4	6	9.5	9.5	—	—	—	—
10	S.O.	F	62	As,EH,AF,Hep Cir	ABBA	15	3	4	6	6	—	—	—	—
11	J.R.	F	41	Rh,MS,MI,EH,AF	BAAB	21.5	5	4.5	7	6.5	—	—	—	—
12	J.R.	F	41	Rh,MS,MI,EH,AF,Hep Cir	ABBA	22	1	1	3.5	3	—	—	—	—
13	L.R.	M	76	As,EH,RSR	BAAB	22.5	2.5	4	5	5.5	—	—	—	—
14	F.S.	M	56	As,Hyp,EH,RSR	BAAB	16.25	3(a)	4.5(a)	6(a)	9(a)	—	—	—	—
15	E.H.	F	52	Rh,MS,MI,AI,EH,AF,Hypopr	ABBA	8.9	2	2	3.8	3.3	—	—	—	—
16	C.C.	M	45	Rh,MS,MI,EH,AF	ABBA	27	3.5	2	6.5	4.5	8	9	—	—
17	S.S.	M	37	Rh,MS,MI,EH,AF	BAAB	26.7	3.7	4.9	6.6	8.2	7.7	11	14.7(b)	11.9
18	N.K.	M	55	Rh,MS,MI,EH,AF	ABBA	43	7	5	10	9	13	11	13	13
19	S.M.	M	44	As,Hyp,EH,RSR	BAAB	33	5.5	5	8	8	10	10	11.5	11.5
20	E.P.	F	65	As,Hyp,EH,AF	ABBA	60	4	4	9	9	13	16(b)	15	23(b)

Edema (difference between initial weight and weight at "dry" level)
As(arteriosclerotic); Hyp(hypertensive); Rh(rheumatic); EH(enlarged heart); Cor Thr(coronary thrombosis)
MS(mitral stenosis); MI(mitral insufficiency); AI(aortic insufficiency); RSR(regular sinus rhythm)
AF(atrial fibrillation); Hep Cir(hepatic cirrhosis); Hypopr(hypoproteinemia)
A(Standard) and B(Test-drug) are the same material
(a) febrile reaction to the mercurial; (b) 1 high value of 7 lbs.

this to emphasize the fact that valid comparisons are possible by this method in the course of routine treatment. The patients were unselected except for the fact that they supplied 24-hour weight losses for two or more successive daily doses of 2 ml. intramuscular meralluride. The essential part of their management consisted of (1) bed-rest, (2) digitalization, (3) diet of milk and water, 1 to 1.5 L. of each, (4) a daily intramuscular dose of 2 ml. meralluride, and (5) a fairly accurate daily weighing. It may be noted that although the weighing in these was made by personnel alerted to the importance of avoiding errors in the weighings, inaccuracies were not fully prevented. The control of the procedure was more stringent in the present study. The characteristics of the group are summarized in Table 1. The ages ranged from 37 to 80. Both men and women, and the common etiological varieties of heart disease are represented. The amount of edema ranged from 5 to 60 lbs. Some supplied only one pair of doses, others two pairs, and a few, three and four pairs. The table shows the 24-hour weight losses which are the cumulative values in the case of more than one pair. One member of a pair was designated the "standard" and the other the "test-drug" in accordance with the pattern assigned to each patient.

For an impression of the number of patients and pairs likely to be needed in an assay, the data were distributed arbitrarily in seven groups (Table 2).

TABLE 2.—COMPARISON OF MERALLURIDE 2 ML. I.M. (STANDARD S) WITH ITSELF (TEST-DRUG T) IN BED-PATIENTS IN CONGESTIVE FAILURE

*Groups**	*Edema (lbs.)*		*Average T/S*	*C.I. p.95*
	Average	*Range*		
I. All 20 patients (first pair)	21.4	5–60	108.1	94.1, 122.1
II. 7 patients who supplied only 1 pair	13.9	5–30.5	95.8	74.5, 117.1
III. First 6 patients with 2 pair	24.5	13.5–43	97.2	78.4, 116.0
IV. Next 7 patients with 2 pair	26.3	8.9–60	103.6	83.0, 124.2
V. All 13 patients with 2 pair	25.5	8.9–60	100.7	88.8, 112.6
VI. 5 patients with 3 pair	37.9	26.7–60	115.8†	84.9, 146.7
VII. 4 patients with 4 pair	40.7	26.7–60	108.4	58.6, 158.2

*Based on data in Table 1.
†One high value of 7 lbs.

In the 13 patients with two pairs (4 doses), the T/S ratio obtained was 100.7 (5% confidence limits of 88.8 and 112.6), the expected value being 100. Satisfactory agreement was seen with even smaller groups of 6 and 7 patients with two pairs of doses. As to be expected, the precision of the values declined as the number of patients in a group becomes smaller.

For the second challenge, the 2-ml. dose of meralluride (standard) was compared with the 1-ml. dose (test-drug) in another group of 9 patients. The treatment was the same except that most of these received a low salt diet

TABLE 3.—COMPARISON OF MERALLURIDE 2 ML. I.M. (Standard) with 1 ml. (Test-drug) in Bed-Patients in Congestive Failure

Case No.	*Pattern*	*Edema (lbs.)*	*Average 24-hr. wt. loss per dose in 2 pair (lbs.)*		*Efficacy of Test-drug T/S*	*Analysis based on signs*		
			Standard (S)	*Test-drug (T)*		*Number of paired Comparisons*		*Patients in whom Standard better (+) or Test-drug better (−)*
						Standard better	*Test-drug better*	
1	BAAB	25.5	4.5	2.25	50.0	2	0	+
2	ABBA	19.5	2	1.75	87.5	1	1	0
3	BAAB	14.5	4.75	0.5	10.5	2	0	+
4	ABBA	11	3.25	1.5	46.1	2	0	+
5	BAAB	22	3.25	2	61.5	2	0	+
6	ABBA	32.5	7	4.25	60.7	2	0	+
7	BAAB	10.5	1.75	1.75	100	1	1	0
8	BAAB	29	5.5	3.0	54.5	2	0	+
9	BAAB	22.5	4.5	3.75	83.3	1	1	0
				Average:	61.6	Totals: 15	3	6(+), 0(−)
						Sign test: 6.72		Sign test: 4.16

Efficacy of Test-drug 61.6% of Standard with Confidence Interval 41.2, 82 at 5%.

(approximately 2 gm. salt) instead of the milk and water, and in all but one (Case No. 6) the drug was given every other day. The results are summarized in Table 3. The effect of the 1-ml. doses was 61.6% of that of 2 ml. (5% confidence limits of 41.2 and 82). This is similar to the ratio of 1- and 2-ml. doses in the dose-response curve of the previous study with ambulant patients (Fig. 3). A larger number of cases would be expected to improve the precision of the efficacy value.

As already mentioned, since the weight losses for the ratio T/S are measured in similar periods of time for both drugs, the reverse ratio S/T provides an estimate of the difference in time it would take to clear the edema with each drug alone.

ANALYSIS BASED ON SIGNS. We investigated the possibility of a simplified analysis of the data based on the sign test. The sign test approach has the advantage that it is not involved with restrictive assumptions regarding the structure of the data, as for example, the stability of the paired responses throughout the falling edema curve. In the case of the sign test, for each paired comparison we consider only the direction of the difference in the two responses, not the amount of the difference. This makes it possible to use data from patients with only a few pounds of edema, for in them the weaker agent shows no weight loss, while the stronger continues to show a loss. It has the added advantage that the calculations in the test for significance are sometimes so simple that one can make them at a glance without paper and pencil.

The sign test approach is illustrated in Tables 3, 4, and 5, which may be compared with the method using ratios. In symbols, the corrected sign test (Mosteller[15]) is $\frac{(X-Y-1)^2}{X+Y}$. Thus in Table 3, among the total of 18 pairs, the standard produced more weight loss than the test agent in 15 pairs ($X=15$) and in 3 pairs ($Y=3$) the direction was reversed. We subtract 3 from 15 ($15-3=12$) and then subtract 1 more as the "correction term" ($12-1=11$). We square the result ($11^2=121$), divide by $X+Y$ (18) and obtain the number 6.72. If the final number exceeds 3.84, the difference is significant at the 5% level.

It may be noted that in the 13 patients in whom the standard and test-drug were the same (Group V of Table 2), the sign test confirmed the fact by failing to discriminate between them. Applied in terms of the 13 patients, it yielded the number 0.17; and in terms of the 26 paired comparisons, zero.

Either one or the other form of analysis may be employed. As mentioned above, the sign analysis will indicate only which is the more effective agent, while the ratio analysis gives in addition an estimate of the amount of the difference. The use of both tests may sometimes provide more information than either alone, as, for example, the order of significance by the sign test aiding in the interpretation of borderline discriminations by the ratio method. There is also the chance of disagreement by the two tests which may call attention to a possible abnormality in the sample of test-patients or to the possibility that some particular diuretic calls upon a mechanism which operates in some patients and not in others, as in the case of aldosterone-blocking agents. Experience may help decide how to manage in the particular situations in which the two tests may disagree.

ASSAY OF TWO ORAL DIURETICS. In the course of this study on the method, an opportunity presented itself to examine two oral diuretics with a few test-patients for each. These results

TABLE 4.—PRELIMINARY ASSAY OF CHLOROTHIAZIDE (DIURIL).

Case No.	*Pattern*	*Edema (lbs.)*	*Average 24-hr. wt. loss per dose in 2 pair (lbs.)*		*Efficacy of Test-drug T/S*	*Analysis based on signs*		
			Standard (S)	*Test-drug (T)*		*Number of paired Comparisons*		*Patients in whom Standard better (+) or Test-drug better (−)*
						Standard better	*Test-drug better*	
1	ABBA	7.0	1.25	0.5	40.0	2	0	+
2	BAAB	10.5	1.75	1.0	57.1	2	0	+
3	ABBA	7.5	1.25	0.5	40.0	2	0	+
4	BAAB	23.0	4.0	3.5	87.5	1	0	+
5	ABBA	7.5	1.5	0.5	33.3	2	0	+
6	BAAB	19.0	1.5	0.25	16.7	2	0	+
7	ABBA	7.0	2.25	1.25	55.6	2	0	+
				Average:	47.2	Totals: 13	0	7(+), 0(−)
						Sign test: 11.07		Sign test: 5.14

Standard (meralluride i.m. 2 ml.); Test-drug (chlorothiazide oral 2 gm.)
Sign test value exceeding 3.84 significant at 5%
Efficacy of Test-drug 47.2% of Standard with Confidence Interval 26.4, 68 at 5%.

TABLE 5.—PRELIMINARY ASSAY OF A PHENOTHIAZINE COMPOUND

Case No.	*Pattern*	*Edema (lbs.)*	*Average 24-hr. wt. loss per dose in 2 pair (lbs.)*		*Efficacy of Test-drug T/S*	*Analysis based on signs*		
			Standard (S)	*Test-drug (T)*		*Number of paired Comparisons*		*Patients in whom Standard better (+) or Test-drug better (−)*
						Standard better	*Test-drug better*	
1	ABBA	12.5	2	0.75	37.5	2	0	+
2	BAAB	20.0	1.5	1.0	66.7	1	1	0
3	ABBA	12.0	2.75	1.5	54.5	2	0	+
4	BAAB	12.0	1.5	0.75	50.0	2	0	+
5	ABBA	30.0	1.75	1.0	57.1	2	0	+
6	BAAB	13.0	1.75	0.75	42.9	2	0	+
7	BAAB	15.5	1.25	0.25	20.0	2	0	+
8	BAAB	16.5	4.5	2.25	50.0	2	0	+
				Average:	47.3	Totals: 15	1	7(+), 0(−)
							Sign test: 10.56	Sign test: 5.14

Standard (meralluride i.m. 2 ml.); Test-drug (a phenothiazine U5641 oral 1 gm.)
Efficacy of Test-drug 47.3% of Standard with Confidence Interval 35.5, 59.1 at 5%.

show only preliminary, not definitive values for their efficacy. For the latter it would be desirable to increase precision of the answer by the inclusion of more patients.

Table 4 shows the application of the method to chlorothiazide (Diuril) in 7 patients. The 2-gm. oral dose produced 47.2% of the effect of the 2-ml. intramuscular dose of meralluride with confidence limits of 26.4 and 68 at the 5% level.

Table 5 shows its application to an experimental phenothiazine compound which is not in therapeutic use, in a group of 8 patients. The 1-gm. oral dose produced 47.3% of the effect of the 2-ml. intramuscular dose of meralluride with confidence limits of 35.5 and 59.1 at the 5% level.

Discussion. The belief is fairly general that the final decision concerning the efficacy of a new diuretic agent must come from comparisons made in man. The meager supply of accurate information from this source has been due largely to want of satisfactory methods. Various types of test-subjects, of responses, and of experimental designs have been tried. Lipschitz and Stokey[13] suggested the measure of salt and water excretion by normal persons receiving the diuretic together with a volume of salt solution. Clark and Hagans[1] also suggested nonedematous or normal persons on a diet with large amounts of water without salt restriction, and as measures of response, changes in urine volume and body weight. Ford *et al.*[4,5] in a series of studies used nonedematous hospitalized patients who had recovered from congestive failure, and as a measure of response the 24-hour urinary sodium excretion with a diet containing 50 mEq. sodium. Others have also attempted to compare diuretic agents in terms of urinary water and electrolyte excretion in normal persons and in patients in congestive failure (Kattus, Arrington and Newman[12], Spencer and Lloyd-Thomas[16]).

Upon close examination of the various reports, one finds disagreements in estimates of diuretic potency of several agents, which may be due to many factors including the type of test subject, the response that was measured, and the experimental design. Several examples may be cited. Kattus, Arrington and Newman[12] found 1 gm. of the pyrimidinedione mixture #2614 equal to 2 ml. meralluride in normal persons, but weaker in patients with edema. We found it extremely weak by our previous method of assay, 1 gm. equal to only 0.36 ml. meralluride (Greiner *et al.*[9]). Ford *et al.*[4] found 1.2 gm. aminometradine nearly equal to (about 85%) 2 ml. meralluride, while our former assay in ambulant patients assigned to it a value of less than 1 ml. They also assigned to 2 ml. mercaptomerin a value of only about 58% of 2 ml. meralluride, while by the method we previously used (Greiner[10]), the two were indistinguishable. Another case of disagreement between the results with our previous method and that of Ford *et al.* is described in the paper by Greiner *et al.*[9]. There in a comparison of two compounds belonging to the same group, the pyrimidinediones, aminometradine and amisometradine, the results by the two methods were in close agreement, but in the comparison of these with a compound of another group, meralluride, the results differed widely. They used hospitalized patients who had recovered from congestive failure and sodium excretion as the response, while we used ambulatory patients in congestive failure and body weight loss as the response. There are, to be sure, other possible explanations for the discrepancies.

The various reports mentioned above show that the measure of urine volume

may also yield different answers from that of sodium excretion in the same person. The present study further indicates that the patient with little or no edema is not very sensitive and usually quite erratic in response to a series of doses of a diuretic.

It has been our position that quantitative evaluations directly applicable to therapeutic practice would probably have to come from patients in congestive failure, in spite of the warning which many workers have emphasized, namely, that such patients present formidable difficulties for the comparison of diuretic agents (Clark and Hagans[1], DeGraff, Nadler and Batterman[3], Greiner[10], Greiner and Gold[11], Kattus, Arrington and Newman[12], Spencer and Lloyd-Thomas[16]). We also believe that a measure of body weight loss, being a comprehensive expression of edema loss, avoids a possible source of misinformation in a comparison of agents which may have selective activity on the excretion of urinary components, water and electrolytes, and should therefore provide a more stable index of the relative efficacy of diuretics in the matter of clearing edema. There is need of a thorough investigation of this point. DeGraff, Nadler and Batterman[3], comparing diuretics in hospitalized patients in congestive failure, observed that changes in body weight as a measure showed less fluctuation than urine output.

In an early report from our clinics (Modell, Gold and Clarke[14]), we used ambulant patients in moderate congestive failure and, as a measure, the 15-hour weight loss in a comparison of diuretics at three dose levels. We were unable to distinguish the two test agents but there was also the fact that the method was crude, not sufficiently sensitive to distinguish 1 ml. from 2 ml. of the same drug with 125 doses in a group of 39 patients. The major technical defects were largely overcome and a useful method for the bio-assay of diuretic agents in ambulant patients in moderate congestive failure was developed (Greiner *et al.*[8]) with the 24-hour weight loss as the measured response, a system of randomization of drugs and doses in one and the same patient to deal with variables, and dose-response curves for comparative results suitable for computation of potency with confidence limits. This assay is conveniently performed in a cardiac clinic with a group of about 25 ambulant patients. In the case of oral diuretics, the trial of the largest doses feasible without an excessive incidence of unpleasant local effects provided a practical therapeutic expression in the evaluation of these agents, since there is little merit in a compound of high diuretic potency only in doses that are precluded by toxic effects (Greiner and Gold[11]). We have employed this method successfully in large numbers of assays which have been published during the past 8 years, the most recent (Greiner *et al.*[9]) describing its application in the earliest screening operation of a new group of diuretic agents as their development took place.

The method now described in the present report puts to use bed-patients in varying degrees of advanced congestive failure. It differs from our previous method in several respects. The 24-hour weight loss responses are here not measured from a constant weight base, but from the slope of a falling weight base, the effect of the falling base being distributed between the two drugs. Our former method yields two kinds of information. One is an expression of relative diuretic "potency," the amounts of the standard and test-drug required to produce similar effects. This expression does not necessarily reveal that one agent is

preferable to another, for, with this information alone, all that is necessary to secure the same result is to give more of the weaker drug. The other, obtained in the case of the oral diuretics, is an expression of practical utility which points to the agent that is preferable, since the comparison is made in terms of the largest doses without an undue incidence of side-effects. The present method gives only the second kind of information. It supplies data only on "ceiling effects." It does so in two ways: by the diuretic (pharmacologic) ceiling doses in the case of compounds with low toxicity and by the practical (therapeutic) ceiling doses in the case of agents with sufficient toxicity to preclude the trial of doses having the maximal diuretic effects.

In the comparison of the standard (2 ml. meralluride) with the same drug in one-half dose, the ratio of efficacy proved to be similar to the ratio of the two from the dose-response curve in ambulant patients with the former method. Since the principle in establishing relative "potency" with dose-response curves involves the use only of the steep or sensitive part of the curve, while the present method calls for doses only at or above the ceiling for an expression of "efficacy," it is anticipated that, in the case of drugs with different modes of action, the results by the two methods will not always be the same.

The new method has several advantages. Since it does not call for accurate dose-response curves, it requires fewer patients. After a few preliminary trials to ascertain the doses at or above the "ceiling," an assay may be completed in a much shorter period of time, about 10 days as against 4 to 6 weeks. It eliminates the need for deciding whether a particular patient in an assay might be placed at a disadvantage by the interval of one week between doses for the return of the weight to the original level. The results of an assay emerge out of the framework of a standard regimen of treatment for congestive failure.

We are now applying the method to the assay of various diuretic agents. A larger experience may suggest modifications and revisions with respect to details, such as the kind of standard for comparison, the number and qualifications of test-patients, the base-regimen, selection and number of paired comparisons, intervals between doses, the period of the measured response, and the interpretation of the statistical analyses. The design may need to be altered to adapt it to the special properties of some diuretic agents.

Summary. 1. The details of a new method are described for the evaluation of diuretic agents using bed-patients in congestive failure.

2. A test-drug is assayed against a standard by paired comparisons of a series of doses of the two agents given concurrently to the same patients, the doses used being at the "ceiling" level for each drug.

3. The measured responses are the 24-hour weight loss per dose.

4. The diuretic efficacy of a test-drug is computed in relation to the standard from an analysis of the data in terms of the mean of the ratios of responses in several patients with confidence limits for significance, and by the sign test.

5. The competence of the method for the assay of diuretics was challenged by a comparison of meralluride with itself, which yielded the expected value with a high degree of precision; also, by a comparison of meralluride with itself in one-half the dose, which yielded a ratio similar to that obtained with our previous method using dose-response curves.

6. Since the comparisons are made

with doses which represent the maximum therapeutic potentiality of each agent, the results of an assay reveal which of the two agents is the more effective for clearing the edema, how much more effective it is, or how much more quickly it may be expected to bring about the therapeutic result.

7. The new method has the advantages that it is simple to perform, yields dependable answers rapidly, requires relatively few patients for an evaluation and is carried out within the framework of a highly effective therapeutic regimen for congestive failure.

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SUMMARIO IN INTERLINGUA

Un Rapide Methodo Quantitative pro le Comparation de Agentes Diuretic in Patientes Allectate con Disfallimento Congestive

1. Es describite le detalios de un nove methodo pro le evalutation de agentes diuretic, con le uso de allectate patientes in disfallimento congestive.

2. Le droga-candidato es essayate contra un standard cognoscite per comparationes appareate de un serie de doses del duo agentes que es administrate in alternation al mesme patientes. Le doses usate in ambe casos es le doses de efficacia maximal.

3. Lo que es mesurate es le perdita de peso in 24 horas in responsa a un dose individual.

4. Le efficacia diuretic del droga-candidato es computate in relation al standard ab un analyse del datos in le forma del media del proportiones del responsas in plure patientes, con limites de confidentia in le signification, e per le test del signo.

5. Le competentia del method pro le essayage de diureticos esseva ponite al proba per un comparation de merallurido con se ipse. Iste test produceva le

expectate valor con un alte grado de precision. Merallurido esseva etiam comparate con se ipse in un dose reducite al medietate. Iste test produceva un proportion simile a illo obtenite con nostre previe methodo de curvas de dose e responsa.

6. Viste que le comparationes es facite con doses representante le maximo del potentialitate therapeutic de omne agente individual, le resultatos revela le qual inter duo agentes es le plus efficace in reducer un edema, a qual grado illo es plus efficace, o quanto plus rapidemente illo pote producer le expectate effecto therapeutic.

7. Le nove methodo ha le avantages que illo es simple a executar, que illo produce rapidemente responsas fidel, que illo require relativemente pauc patientes, e que su effectuation se face intra le quadro de un efficacissime regime therapeutic pro disfallimento congestive.

(Continued from page 744)

calcification, le rolo de magnesium, e alterationes del systemas enzymatic mineral. Es suggerite que le alterationes in question es possibilemente initiate per irregularitates del pH del corpore e que istos, de lor parte, es le effecto de un processo de invetulation del renes.

Es discutite le action positive del mentionate agente de chelation in iste serie de evenimentos. Il pare possibile que le action include le restauration de un normal balancia de calcium e magnesium e le stabilisation de essential systemas enzymatic mineral, a parte le elimination de calcium.

A CONTROLLED STUDY OF TRIMETHOBENZAMIDE (TIGAN),* A SPECIFIC ANTIEMETIC

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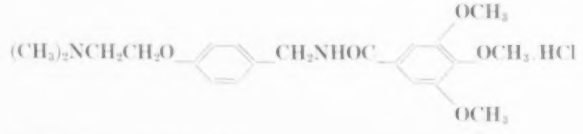
The antiemetic drugs presently employed by the clinician are usually antihistamines, tranquilizers of the phenothiazine group, sedatives, or combinations of these. Therapeutically, many of these medications are relatively effective, but the high percentage of unpleasant side effects sometimes nullifies their usefulness. Consequently the search for more specific and safer antiemetic agents has continued, stimulated in part by the clarification of the vomiting mechanism by Hess[8] and by Wang and Borison[4,17,18]. Wang and Chinn[19] in further studies added to our knowledge of emesis. The vomiting center located in the medulla and the chemoreceptor trigger zone (CTZ) in the floor of the fourth ventricle, dorsolateral to the vagal nuclei, are two known important centers controlling emesis in the central area (Fig. 1).

Recently a number of investigators (Abrams *et al.*[1], Brandman[5], Doyle[6], Nathan[13]) have described the use of trimethobenzamide hydrochloride, a substituted benzamide, in treating disorders accompanied by nausea and vomiting.

Chemically this compound is 4-(2-dimethylaminoethoxy)-N-(3, 4, 5-trimethoxybenzoyl)-benzylamine HCl. Its structural formula is:



Pharmacologically, trimethobenzamide appears to act primarily on the CTZ (Schallek *et al.*[15]). Unlike the phenothiazines, the drug produces no sedation. In laboratory experiments there was no reduction in the capacity of conditioned animals to avoid noxious stimuli, no slowing of EEG activation produced by stimulating the reticular formation electrically, and no central sedation or excitation. The drug does not antagonize the effects of histamine on the bronchial musculature nor depress supramedullary centers, and produces no hypotension. Toxicity studies revealed a wide margin between effective and toxic doses. Pregnant rats, fed daily doses of 100 mg. per kg., showed no congenital anomalies or abortion of litters.

The present investigation to determine the antiemetic efficacy of trimethobenzamide was carried out as a double-blind study.

Procedures and Materials. The group under study comprised a total of 161 patients with nausea and vomiting from a variety of causes including infection, gastrointestinal disturbances, allergy (including drug-induced), toxicosis, hyperemesis gravidarum and others (Table 1). Trimethobenzamide was administered to 95 and a placebo to 66 patients. Medication was given every 6 hours, orally in the form of capsules, intramuscularly or by rectal suppository. The placebo of each

*Hoffmann-La Roche Inc., Nutley, N.J.

form was identical in appearance to the active drug which consisted of 200 mg. of trimethobenzamide hydrochloride. All treatment forms were coded so that neither the investigator nor the patient knew which was received until after treatment. The treatment form was allocated according to the type of disease; except for this, patient selection was random. The number of patients receiving each dosage form is shown below:

Form	*Trimethobenzamide*	*Placebo*
Capsule	49	30
Ampul (I.M.)	26	19
Suppository	20	17
	95	66

All patients received the same amount of drug per dose.

The median age of patients receiving active medication was 26.9 years compared with 25.6 years of the placebo group. Fifty-five per cent of the patients in the experimental group were females, compared with 45% of the placebo-controls. The median duration of symptoms prior to treatment in both groups was 1.2 days.

Patients were observed for evidence of drowsiness, dryness of mouth, dizziness, pulse rate and blood pressure changes following the administration of medication. Rapidity and duration of relief were also determined in each patient.

Patients' symptoms of nausea and vomiting prior to (Table 2) and following (Table 3) treatment were graded as follows: absent, 0; mild, 1; moderate, 2; and severe, 3; the difference in severity formed the basis of evaluation.

Results. Nausea was controlled completely in 58 (61.1%) of all patients receiving active medication, and vomiting in these same patients was absent after therapy in 79 (83.1%). After treatment mild nausea occurred in 29 (30.5%) of the treated group, and mild vomiting in 12 (12.6%). Four (4.2%) patients each had moderate and severe nausea and 1 (1.1%) and 3 (3.2%) moderate and severe vomiting.

None of the placebo patients received complete relief from nausea and

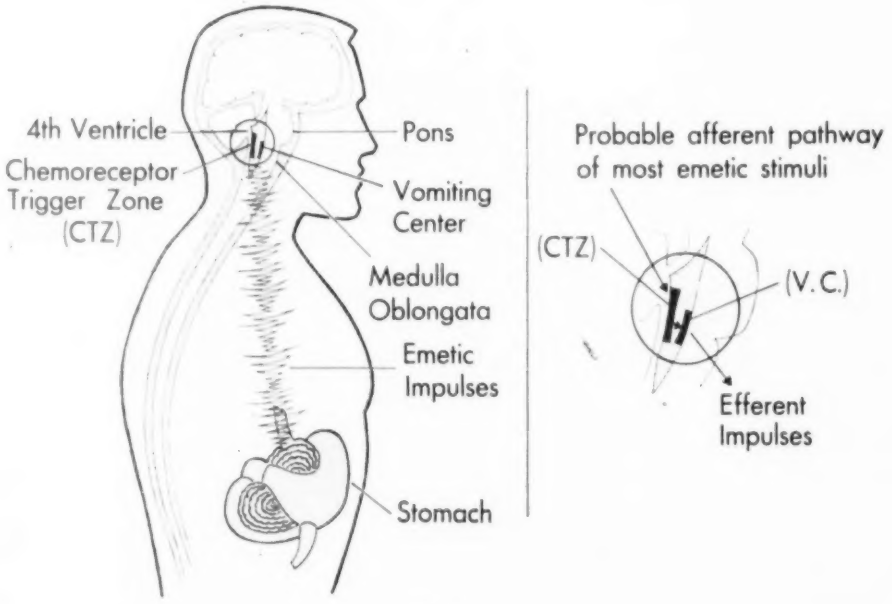


Fig. 1

only 1 (1.5%) noted complete cessation of vomiting. Eight (12.1%) had mild nausea and 19 (28.8%), mild vomiting; 43 (65.2%), moderate nausea and 38 (57.6%), moderate vomiting; and 15 (22.7%), severe nausea and 8 (12.1%), severe vomiting (Table 3).

of the symptoms. No change in symptoms occurred in 5 patients receiving the active antiemetic nor in 54 receiving the placebo (Table 1).

Of the 5 in the trimethobenzamide-treated group who failed to respond to medication, 2 had viral gastritis and 1

TABLE 1.—EFFECT OF MEDICATION ON SYMPTOMS OF NAUSEA AND VOMITING

	Trimethobenzamide (Treated Patients)				*Placebo (Control Patients)*			
Diagnosis	*Number*	*N. and V. Absent*	*N. or V. or Both Present to a Lesser Degree*	*No. Change*	*Number*	*N. and V. Absent*	*N. or V. or Both Present to a Lesser Degree*	*No Change*
Infection	42	27	12	3	31	0	7	24
GI disturbance	13	6	6	1	12	0	2	10
Drug administration or allergy	21	15	6	0	11	0	1	10
Toxicosis	3	1	2	0	2	0	0	2
Hyperemesis gravidarum	5	1	4	0	6	0	2	4
Miscellaneous	11	8	2	1	4	0	0	4
TOTALS	95	58	32	5	66	0	12	54

In summary, nausea and vomiting were absent in 58 of the 95 treated patients but in none of the control patients. Thirty-two patients receiving the active drug and 12 who received the placebo had a decrease in the severity

each, gastroenteritis, alcoholic gastritis and congestive heart failure. This failure of response could not be accounted for by the disease, since other patients with the same syndromes were treated successfully. Table 3 shows the severity

TABLE 2.—THE GRADED SEVERITY OF NAUSEA AND VOMITING PRIOR TO TREATMENT

	Nausea				*Vomiting*			
	Trimethobenzamide		*Placebo*		*Trimethobenzamide*		*Placebo*	
Severity	*Number*	*%*	*Number*	*%*	*Number*	*%*	*Number*	*%*
All Cases	95	100.0	66	100.0	95	100.0	66	100.0
Absent	0	0	0	0	0	0	0	0
Mild	3	3.1	1	1.5	26	27.4	12	18.2
Moderate	63	66.3	40	60.6	55	58.0	44	66.7
Severe	29	30.5	25	30.9	14	14.6	10	15.1

TABLE 3.—THE DISTRIBUTION OF DRUG-TREATED AND PLACEBO-TREATED PATIENTS WITH REGARD TO SEVERITY OF SYMPTOMS AFTER TREATMENT

	Nausea				*Vomiting*			
	Trimethobenzamide		*Placebo*		*Trimethobenzamide*		*Placebo*	
Severity	*Number*	*%*	*Number*	*%*	*Number*	*%*	*Number*	*%*
All Cases	95	100.0	66	100.0	95	100.0	66	100.0
Absent	58	61.1	0	0	79	83.2	1	1.5
Mild	29	30.5	8	12.1	12	12.6	19	28.8
Moderate	4	4.2	43	65.2	1	1.1	38	57.6
Severe	4	4.2	15	22.7	3	3.1	8	12.1

of symptoms following therapy in both groups. There was an almost complete reversal in patient condition before and after treatment in the trimethobenzamide group but very little change in the placebo group. This is illustrated in Fig. 2 by the change in configuration of the graphs from ratings 0 to 3 before and after trimethobenzamide administration, and the similarity in configuration before and after placebo administration.

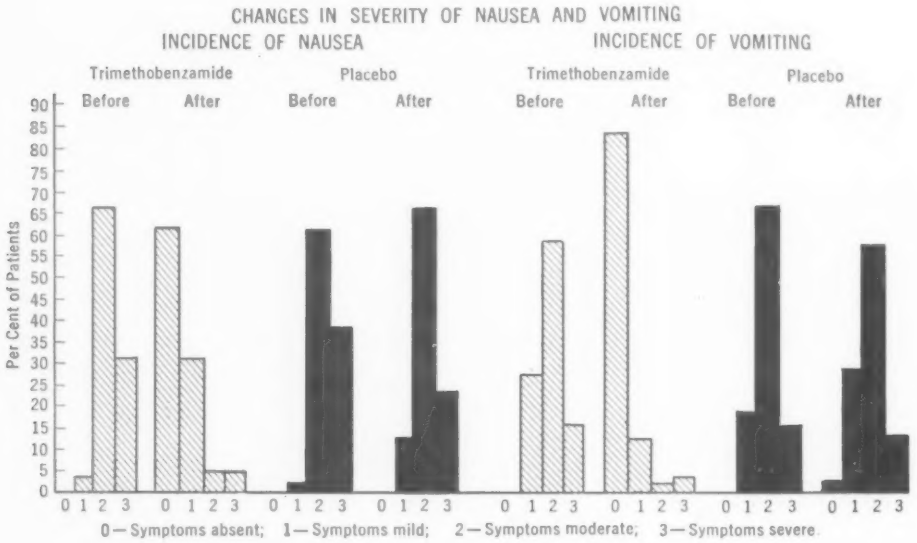


Fig. 2

Onset of relief occurred within an average of 30 minutes following treatment in patients receiving the active medication, and the median duration of relief was between 4 and 5 hours. No significant relief was noted for the control group. No side effects of any type were observed in either group.

Discussion. In studying the effect of a drug on the symptom complex of nausea and vomiting, two predominant questions arise: 1) How long would the condition have persisted without medication? and 2) To what extent are the symptoms due to psychic influences? Conclusions based on uncontrolled observations, even though made in good faith, may well be erroneous. Nor is the comparison of results with those of another group of investigators a reliable method, since the number of variables and extraneous conditions at play are incalculable. Comparing two similar groups, one receiving active medication and the other none, represents an attempt to answer the first question. Administration of a placebo under a double-blind technique should give an indication of the part played by psychic or other factors. This type of study eliminates the observer's bias and the patient's psychic factors.

In designing a double-blind study it is both necessary and important to anticipate the differences that may be apparent between the test drugs so that sufficient data can be collected for substantiation of results. The

greater the similarity in details of the groups under investigation, the more valid the comparison. Thus, although age and sex may appear to be insignificant details, these factors may have some bearing on or even reduce the effect of such uncontrollable or unknown factors, such as patient's temperament or physical and functional state at the time of the experiment. Similarly, the personalities of the investigators may exert an influence (Modell and Houde[11]).

Randomization spreads the chance of error (Modell and Houde[11]). As mentioned previously, randomization in our investigation was not entirely possible. Apart from the unequal number of subjects, the two groups were quite comparable in distribution of age, sex and cause of symptoms. The difference in severity of nausea or vomiting, or both, prior to treatment suggests that the symptoms of patients in the placebo group (as reported by them) were slightly more severe than in those who received trimethobenzamide. However, this difference was not statistically significant. On the other hand, the differences in results of trimethobenzamide- and placebo-treated patients with regard to severity distribution were highly significant, indicating that something other than chance was operating. Yet, the almost complete reversal in the patient condition, before and after treatment in the trimethobenzamide group, makes a true double-blind test situation difficult to maintain. Thus, at the end of the test period the symptoms had not changed appreciably in the control group whereas they were absent in the majority of the treated patients.

From these results it can be assumed that trimethobenzamide is an effective antiemetic in a variety of conditions. To determine how effective it is in comparison with other drugs would require a similar study using one or more active agents.

Although direct comparisons with other studies are untenable for the reasons mentioned, certain qualities can be accepted as characteristic of the drug when they are reported by a large number of observers. Most of the currently available antiemetics have a high incidence of side effects ranging from drowsiness and dryness of the mouth to hypotension and extrapyramidal dysfunction (Knapp and Beecher[9], Moyer[12], Nelson[14]), whereas trimethobenzamide has been free from side effects according to most reporters (Abrams *et al.*[1], Doyle[6], Gauthier[7], Lucinian and Bohn[10], Nathan[13], Shnider and Gold[16]).

Bellville, Bross and Howland[2] designed a method for the clinical evaluation of antiemetic agents. They state that, "The ideal antiemetic agent would be one that completely suppressed nausea and vomiting and produced no untoward side effects." They further remark that complete suppression of vomiting and retching is probably possible but that equal success with nausea, an entirely subjective symptom, is questionable[3]. The incidence of nausea in our study following medication was significantly higher than that of vomiting which was absent in 83.1% of the patients. It is possible that higher doses would have increased the number of positive results. However, one of the above criteria, the absence of side effects, was noteworthy in this as in other reported studies (Abrams *et al.*[1], Doyle[6], Gauthier[7], Lucinian and Bohn[10], Nathan[13], Shnider and Gold[16]).

Summary and Conclusions. To determine the antiemetic efficacy of trimethobenzamide, a double-blind study was conducted in 2 comparable groups of 161 patients (95 receiving trimethobenzamide and 66, placebo) with

nausea and vomiting from a variety of causes.

Following therapy, nausea and vomiting were absent in 58 of the 95 treated patients but in none of the control patients. In 79 receiving the active drug, vomiting had ceased completely but nausea was present to a slight degree in 21 of these. Thirty-two patients receiving the active drug and 12 who received the placebo had a decrease in the severity of the symptoms. No change in symptoms occurred in 5 patients receiving the active antiemetic and in 54 receiving the placebo.

The differences in severity between the trimethobenzamide- and placebo-treated patients were highly significant.

The average onset of relief in the treated group was 30 minutes and the average duration between 4 and 5 hours. No untoward reactions were noted in either group.

In view of the results obtained in this study, trimethobenzamide appears to be a most effective antiemetic. The complete lack of side effects or toxicity and the specific antiemetic property of this new compound make it a valuable addition for the treatment of the distressing symptoms of nausea and vomiting.

It is suggested that further double-blind studies be carried out with several dosages of trimethobenzamide and with other antiemetic agents for comparison.

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SUMMARIO IN INTERLINGUA

Studio Controlate de Trimethobenzamida, Un Antiemetico Specific

Pro determinar le efficacia antiemetic de trimethobenzamida (Tigan), un studio bis-occulte esseva executate in duo comparabile gruppos de (insimul) 161

patientes con nausea e vomito ab un varietate de causas. Novanta-cinque recipeva trimethobenzamida, 66 un placebo.

Post le therapia, nausea e vomito esseva absente in 58 del 95 patientes tractate con le droga sed in nulle del 66 patientes de controlo. In 79 del gruppo a medication active, le vomito habeva disparite completemente, sed leve grados de nausea persisteva in 21 de iste casos. Trenta-duo del patientes in le gruppo de medication e 12 de illes in le gruppo de placebo notava un reduction del severitate de lor symptomas. Nulle alteration esseva manifeste in 5 del medicatos e in 54 del recipientes de placebo.

Le differentias in le grado de severitate del symptomas inter le gruppo tractate con trimethobenzamida e le gruppo recipiente le placebo esseva altemente significative.

Le retardo medie del declaration del alleviamento a partir del momento del administration in le gruppo a medication active esseva 30 minutas. Le persistentia medie del effecto esseva inter 4 e 5 horas. Nulle adverse effectos lateral esseva notate in ulle del patientes del un e del altere gruppo.

Viste le resultatos obtenite in le presente studio, il pare justificate asserer que trimethobenzamida es efficacissime como antiemetico. Le absentia complete de effectos lateral o de toxicitate e le proprietates specificamente antiemetic de iste nove composito face de illo un utile addition al armamentario in le tractamento del enoiose symptomas de nausea e vomito.

Es proponite que studios bis-occulte additional es interprendite con varie dosages de trimethobenzamida e etiam studios de comparation con altere agentes.

(Continued from page 749)

normotensive, sed nulle appreciabile alteration in le nivellos steroide occurreva durante le intervallo standard de tempore ab un collection de specimens de sanguine al altere. Tamen, in subjectos normotensive e etiam hypertensive, un reduction significative occurreva quando le subjecto se teneva in decubito dorsal durante le periodo experimental standard de 1½ horas. Il seque que alterationes positional debe esser prendite in consideration in le interpretation de datos concernite con le nivello de 17-hydroxycorticosteroide del plasma in subjectos human.

THE ACUTE EFFECT OF ETHANOL INGESTION ON PLASMA AND URINARY 17-HYDROXYCORTICOIDS IN ALCOHOLIC SUBJECTS

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In experiments with rats, Smith[11] demonstrated that acute intoxication with ethyl alcohol was accompanied by depletion of ascorbic acid and cholesterol levels in the adrenal cortex, a finding compatible with increased adrenocortical activity. This effect of ethyl alcohol upon adrenocortical function has been corroborated by others in various animal species. Thus, depletion of adrenal ascorbic acid and cholesterol after ethanol in guinea pigs has been reported by Forbes and Duncan[2] and in mice, by Santrestiban and Swingard[9]. To our knowledge, increased adrenocortical function after ethanol ingestion has not been demonstrated in man.

Studies were thus undertaken to determine what changes in adrenocortical function occurred in humans (alcoholic patients and non-alcoholic controls) after ingestion of a measured amount of ethyl alcohol.

Method. A total of 106 alcoholic patients was studied. These comprised males of ages from 21 to 53 years (mean age 38 years) with known histories of chronic alcoholism. They were selected from the wards of Kings County Hospital following admission during an acute alcoholic episode. After about one week's treatment, including vitamin and other nutritional rehabilitation, the patients were transferred to the investigational ward of the William A. White Clinic for biochemical and physiologic study.

Control subjects comprised 15 healthy, non-alcoholic male volunteers, matched roughly for age with the alcoholic patients, and selected from the Hospital Resident staff, the Medical Student body, and from the local civilian population of Brooklyn, New York.

Alterations in adrenocortical function were sought by measurement of changes in urinary and plasma 17-hydroxycorticoids*; urinary 17-ketosteroids* and circulating eosinophils. The 17-hydroxycorticoids were estimated by the method of Silber and Porter[10], and urinary 17-ketosteroids, by that of Callow, Callow and Emmens[1]. Eosinophil changes were measured according to Thorn *et al.*[12].

In all experiments, ethyl alcohol was administered in the form of a standardized beverage. This was prepared in the laboratory before each test to contain 1 ml. of "absolute" ethanol† per kilogram of body weight. This

*Crystalline corticoids used for reference standards were supplied through the courtesy of Dr. J. L. Graeme of Ciba Pharmaceutical Products Inc., Summit, New Jersey.

†Ethyl Alcohol, Publicker Industries Inc., Philadelphia, Pa., USP 200 Proof.

amount of ethanol was added to unsweetened citrus fruit juice such that the final concentration was 25% by volume and designated the "Test Drink." A "Control Drink" of fruit juice without ethanol and having a volume equal to that of the corresponding Test Drink was likewise prepared.

EXPERIMENTAL. (1) *Changes in urinary 17-hydroxycorticoids and 17-ketosteroids 4 hours after ingestion of ethanol.* In this experiment, changes were determined in steroid excretion rate 4 hours after ingestion of the ethanol Test Drink. Eighteen alcoholics and 15 non-alcoholic control subjects were tested. Tests were begun at the same time each morning on 2 consecutive days with the subject fasting and at bed rest. On the first day, the requisite amount of the non-alcoholic Control Drink was ingested immediately after complete voiding and all urine collected for the subsequent 4 hours. This procedure was repeated identically on the second day except that the Test Drink was taken.

In Table 1 are listed the respective excretion rates on the test and control days for the two groups studied. Tests of significance of these data were done using the analysis of variance model. This analysis revealed no significant differences between these groups either in 17-hydroxycorticoids or in 17-ketosteroids following the ingestion of the alcohol Test Drink. As is customary in the analysis of variance procedure, in view of the non-significant treatments by group interaction, the treatment effects, that is, the effects of ethanol, were then tested over all subjects. The

TABLE 1.—CHANGES IN URINARY STEROIDS 4 HOURS AFTER ALCOHOL TEST DRINK AND NON-ALCOHOL CONTROL DRINK IN ALCOHOLIC PATIENTS AND NON-ALCOHOLIC CONTROLS.

Alcoholic Patients					*Non-Alcoholic Controls*				
	17-Ketosteroids (mg./hr.)		*17-OH Corticoids (mg./hr.)*			*17-Ketosteroids (mg./hr.)*		*17-OH Corticoids (mg./hr.)*	
Patient Designation	*Control*	*Test*	*Control*	*Test*	*Subject Designation*	*Control*	*Test*	*Control*	*Test*
1. N.McL.	.20	.31	.23	.35	1. R.G.	.32	.26	.42	.52
2. S.B.	.42	.13	.13	.36	2. L.G.	.49	.29	.32	.29
3. N.C.	.11	.55	.15	.09	3. A.G.	1.15	.18	.32	.49
4. E.O.	.10	.28	.27	.31	4. A.T.	.41	.66	.21	.26
5. N.H.	.29	.32	.27	.23	5. S.H.	.58	.39	.86	1.00
6. K.D.	.46	.26	.17	.37	6. E.J.	.59	.14	.14	.08
7. N.O.	.38	1.25	.09	.42	7. I.D.	.27	.32	.46	.32
8. J.S.	.38	.13	.14	.33	8. N.K.	.37	.37	.45	.32
9. A.McN.	.22	.14	.12	.37	9. F.S.	.33	.57	.22	.35
10. A.M.	.40	.40	.14	.35	10. L.S.	.38	.28	.11	.41
11. T.L.	.44	.26	.35	.32	11. O.T.	.80	.46	.32	.34
12. I.B.	.53	.47	.48	.66	12. S.W.	.46	.37	.06	.22
13. T.W.	.59	.43	.24	.35	13. N.C.	.60	.51	.46	.52
14. S.A.	.60	.52	.35	.34	14. N.O.	.30	.37	.28	.62
15. O.A.	1.00	.39	.25	.28	15. R.T.	.46	.50	.10	.40
16. D.R.	.70	.51	.42	.46					
17. G.C.	.30	.39	.11	.43					
18. D.L.	.21	.27	.42	.48					
Mean Values	.41	.39	.24	.36		.50	.38	.32	.41

resulting analysis disclosed a significant rise in 17-hydroxycorticoids within 4 hours after ingestion of the Test Drink. No significant effect was found in 17-ketosteroids during this interval.

(2) *Serial changes in urinary 17-hydroxycorticoids over 24 hours after ethanol ingestion.* Having demonstrated a significant increase in 17-hydroxycorticoid excretion rate over 4 hours after ethanol, the previous experiment was extended over a period of 24 hours with serial analyses to discover the time and duration of this change.

Seven alcoholic patients were tested on 2 consecutive days. Control and Test Drinks were given as before and all urine collected for 24 hours thereafter at intervals of 0 to 4 hours, 4 to 8 hours, and 16 to 24 hours. Patients were tested in the fasting state during the initial 4 hours. In the course of the

TABLE 2.—SERIAL URINARY 17-HYDROXYCORTICOIDS (mg./hr.) AT INTERVALS AFTER ALCOHOL TEST DRINK AND NON-ALCOHOL CONTROL DRINK IN ALCOHOLIC PATIENTS

	Hours After Ingestion of Drink							
	0–4		*4–8*		*8–16*		*16–24*	
Patient Designation	*Control*	*Test*	*Control*	*Test*	*Control*	*Test*	*Control*	*Test*
1. A.S.	.07	.46	.34	.22	.23	.18	.19	.24
2. T.C.	.36	.45	.31	.18	.21	.25	.24	.16
3. B.F.	.32	.42	.24	.04	.26	.16	.23	.14
4. R.S.	.08	.20	.26	.36	.16	.09	.18	.20
5. W.C.	.04	.30	.10	.17	.20	.10	.10	.16
6. F.V.	.26	.27	.32	.17	.33	.09	.28	.14
7. C.C.	.29	.23	.15	.08	.24	.20	.10	.13
Means	.20	.33	.24	.17	.23	.15	.19	.17

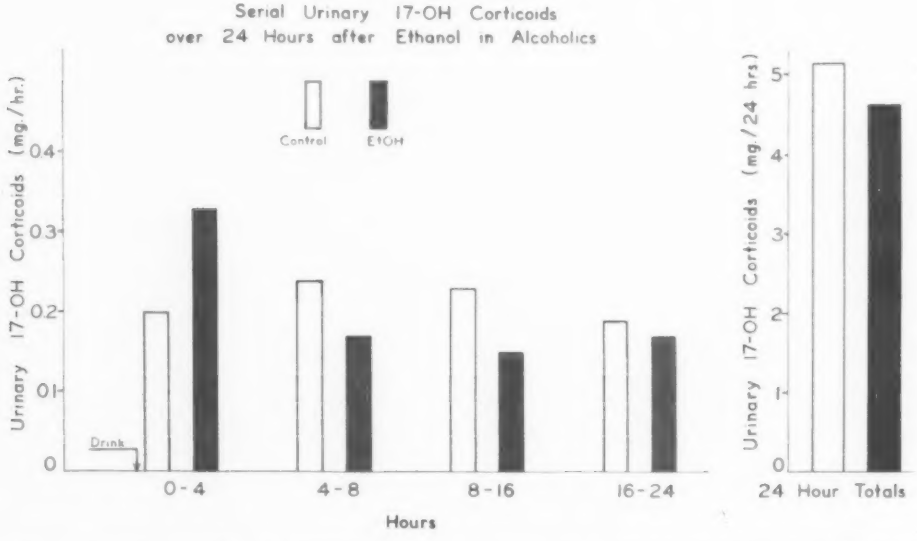

Fig. 1.—Serial changes in excretion rate of urinary 17-hydroxycorticoids over 24 hours following ingestion of ethanol Test Drink and non-ethanol Control Drink in 7 alcoholic patients.

remaining time, they partook of the regular ward dinner and supper and were ambulatory on the ward.

Individual serial values for the 17-hydroxycorticoid excretion rates for control and test days are tabulated in Table 2. Mean values for the respective intervals are shown graphically in Fig. 1.

From these data it is seen that the steroid excretion rate over the 0 to 4-hour interval is greater after ethanol ingestion than after the control drink (confirming the previous experiment). The values then subside to levels somewhat lower than those of their respective controls which show relatively little change throughout. The increased values during the initial 4 hours, however, are not sufficient to cause an increase over 24 hours. In fact, the total 24-hour amount of steroid excreted after ethanol is somewhat less, though not significantly so, than that seen in the control without ethanol. It is thus apparent that following the ingestion of ethanol, the major change in 17-hydroxycorticoid excretion rate is the rise which occurs during the first 4 hours.

(3) *Serial changes in hourly urine volume and 17-hydroxycorticoid excretion rates over 6 hours after ethanol ingestion.* In an attempt further to elucidate the time at which the steroid response to ethanol occurs, and how this relates to urine excretion rate, hourly urine specimens were collected over a period of 6 hours after ingestion of the alcohol Test Drink. Sixteen alcoholic patients were tested, fasting and at bed rest through the first 4 hours; the test at 6 hours was done 2 hours after the usual midday meal. Base-line values were obtained from urine samples collected over one hour prior to ingestion of the alcohol Test Drink.

In Table 3 are listed the serial hourly changes in 17-hydroxycorticoid excretion rates together with the corresponding urinary volume flow over the 6-hour experimental periods. The course of changes in mean values is shown in Fig. 2.

These data show that after ethanol, urinary 17-hydroxycorticoids reach their maximum excretion rate during the first hour and subside thereafter to the lowest levels during the fifth and sixth hours. The one-hour mean (0.55 mg. per 100 ml.) is significantly higher than the resting value (0.32 mg. per 100 ml.) at better than the 1% level (Student's t test of significance of the difference between correlated means). A noteworthy parallelism is apparent between the respective excretion rates of urine and of steroid (Fig. 2). This is suggestive of some participation of renal function in the course of changes observed.

(4) *Serial changes in plasma 17-hydroxycorticoids over 6 hours after ingestion of ethanol.* Following upon the findings with respect to urinary changes after the ingestion of ethanol, a similar experiment was conducted to discover the pattern of changes in 17-hydroxycorticoid levels in the plasma.

Twenty-one alcoholic patients were tested under the same conditions as in the previous experiment. Blood samples were drawn from alternate antecubital veins immediately before, then 2, 4 and 6 hours after ingestion of the alcohol Test Drink. Examinations were made at 2-, rather than at one-hour intervals since the amount of blood required for 6 analyses in each patient was considered prohibitively large. Ten alcoholic patients were similarly studied after ingestion of the Control Drink.

Individual findings and group mean values are listed in Tables 4 and 5. The significance of changes were evaluated by use of Student's t test between correlated means. These data show that

TABLE 3.—HOURLY URINARY 17-HYDROXYCORTICOIDS (mg./hr.) AND URINE VOLUMES (ml. hr.) AFTER ETHANOL INGESTION IN ALCOHOLIC PATIENTS

	Pre-Ethanol —1 to 0 hrs.		*Hours After Ethanol Ingestion*											
			1		*2*		*3*		*4*		*5*		*6*	
Patient Designation	*17-OH*	*Vol.*	*17-OH*	*Vol.*	*17-OH*	*Vol.*	*17-OH*	*Vol.*	*17-OH*	*Vol.*	*17-OH*	*Vol.*	*17-OH*	*Vol.*
1. Lom.	—	—	0.44	220	0.28	510	0.38	150	0.63	65	0.14	25	0.19	25
2. McC.	—	—	0.20	58	0.23	108	0.29	66	0.16	72	0.13	20	0.09	8
3. Wal.	—	—	1.04	510	0.56	255	0.25	64	0.16	33	0.18	28	0.19	21
4. Gib.	0.16	48	0.17	50	0.15	95	0.20	100	0.35	160	—	—	—	—
5. Gl.	0.25	103	0.48	250	0.35	455	0.45	470	0.22	145	—	—	—	—
6. Eth.	0.36	175	0.41	450	0.93	733	0.82	135	0.54	60	0.46	47	0.27	25
7. Cro.	0.46	158	0.75	450	0.22	154	0.20	74	0.23	52	0.16	24	0.17	29
8. Wilm.	0.28	95	0.52	524	0.21	235	0.56	285	0.10	35	0.22	45	—	14
9. Cle.	0.20	52	0.54	480	0.27	205	0.48	120	0.07	22	0.12	28	0.15	25
10. Mar.	0.27	220	0.52	285	0.18	335	—	—	—	—	—	—	0.20	225
11. Dav.	0.39	118	0.47	643	0.35	249	0.42	240	0.32	155	0.12	35	0.35	60
12. O'Br.	0.70	275	1.60	450	0.28	245	0.16	60	0.07	25	0.13	37	0.09	19
13. Mal.	0.24	28	0.24	225	0.19	295	0.16	240			—	—	0.12	18
14. Nev.	0.31	88	0.71	625	0.15	166	0.13	61	0.15	26	0.12	26	0.13	26
15. Wil.	0.13	50	0.13	55	0.23	195	0.32	194	0.12	85	0.17	90	0.10	25
16. Far.	0.36	51	0.56	568	0.09	430	0.14	130	0.21	50	0.39	60	0.11	55
Means	0.32	112	0.55	365	0.29	291	0.33	159	0.24	70	0.20	39	0.17	41

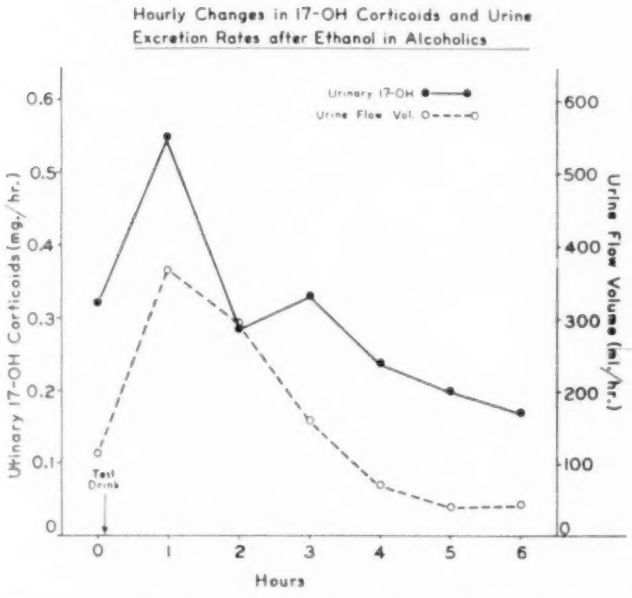

Fig. 2.—Serial changes in hourly urine flow and 17-hydroxycorticoid excretion rates over 6 hours after ingestion of ethanol Test Drink in 16 alcoholic patients.

TABLE 4.—SERIAL CHANGES IN PLASMA 17-HYDROXYCORTICOID LEVELS (μg./per 100 ml.) AFTER INGESTION OF ETHANOL TEST DRINK

Patient Designation	*Pre-Ethanol 0 Hrs.*	*Hours after Ingestion of Ethanol*		
		2	*4*	*6*
1. Qua.	22.6	16.1	26.4	24.9
2. O'Ro.	12.7	2	21.8	—
3. Raf.	14.0	10.3	35.6	14.4
4. And.	25.8	16.8	43.4	21.8
5. Bat.	14.4	2	19.8	11.5
6. Kel.	17.6	12.8	13.2	20.4
7. Lom.	21.0	5.8	—	26.8
8. McC.	10.4	2	—	12.8
9. Wal.	10.8	7.9	10.6	6.3
10. Eth.	26.2	27.5	35.2	21.6
11. Wilm.	23.9	10.7	19.7	16.8
12. Mat.	17.4	21.1	22.1	18.2
13. Dav.	12.2	6.7	6.2	3.5
14. O'Bt.	19.0	10.0	12.0	22.1
15. Nev.	21.9	14.0	12.7	16.9
16. Mal.	11.6	10.5	11.0	10.4
17. Cow.	30.2	25.2	23.1	22.6
18. Klez.	20.7	11.6	12.9	18.5
19. Cog.	11.9	2	7.3	—
20. Wils.	28.2	17.0	20.6	22.2
21. Fat.	19.6	17.3	15.3	13.1
Means	18.7	11.6	19.4	17.1

within 2 hours after the ingestion of ethanol, there is a decrease in the concentration of 17-hydroxycorticoid in the plasma. This is followed by a rapid return to base-line values at the 4- and 6-hour intervals. After the non-alcohol Control Drink, the level does not change significantly at the 2-hour level but falls significantly (p less than .05) at the 4-hour level (Fig. 3). Consequently, neither the fall at 2 hours after the ingestion of ethanol nor the rise at 4 hours can be attributed to diurnal variations of 17-hydroxycorticoid levels in the plasma.

(5) *The relationship between urinary and plasma changes after ethanol: Effects of diuresis.* In Fig. 3 are shown, combined, the changes in 17-hydroxycorticoid levels in plasma together with rates of urine flow and of 17-hydroxycorticoid excretion at 2-hour intervals following alcohol ingestion as described above. The values for urine flow and corticoid excretion rates are the means of two respective one-hour values taken from Experiment 3 and combined to correspond to the 2-, 4-, and 6-hour intervals at which plasma corticoids were measured.

From this figure it is clear that over the first 4 hours after ethanol, plasma corticoid levels vary inversely as the corticoid excretion rate. Further, there can be seen a direct variation between urine flow volume and corticoid excretion. This latter relationship can best be explained in terms of diuresis due to ethanol. Lloyd[5], Kalant[3], and others (Lazo-Wasern and Hier[4], Marks and Leaf[6]) have demonstrated a linear relationship between urine flow volume and 17-hydroxycorticoid output in humans and in laboratory animals. This diuretic effect might also account for the decrease in plasma corticoid level seen at the 2-hour interval, an effect not previously demonstrated. Although the steroids in the plasma are predominantly hydrocortisone, those of the urine, as measured by our methods, contain in addition tetrahydrocortisone and other steroid homologues. Because of this, the data of Experiment 4 cannot be used to demonstrate a direct quantitative relationship between changes in plasma and urinary hydrocortisone. There is, however, the possibility that increased hydrocortisone excretion is one of the factors contributing to the overall rise in urinary corticoids. If this were so, then the fall in plasma hydrocortisone at 2 hours might be attributable to an increased renal clearance. In any event, the changes in plasma and urinary steroids appear to be related to the diuretic effect of ethanol. In order to obtain further data on this effect, a study of another type of diuresis, that following water loading, was undertaken.

(6) *Serial changes in plasma and urinary 17-hydroxycorticoids with diuresis induced by the ingestion of a water-load.* Eleven alcoholic patients were tested under the same conditions as described above. Immediately after collection of pre-test base-line urine and blood samples, each patient drank 1500 ml. of tap water over a period of 5 to 10 minutes. Blood and urine samples were then collected at 2-hour intervals for 6 hours.

Serial changes are tabulated in Table 6 with the group means plotted in Fig. 4. The significance of changes were evaluated by use of Student's t test between correlated means. From these data it is seen that for the initial 2 hours the changes in plasma and urinary corticoids after water diuresis follow the same pattern as those previously found after ethanol. It thus would seem that after ethanol the increased rate of excretion of 17-hydroxycorticoids and the decreased level of these steroids in the plasma both might be due to an increase in the renal clearance of these

TABLE 5.—SERIAL CHANGES IN PLASMA 17-HYDROXYCORTICOID LEVELS (μg. per 100 ml.) AFTER INGESTION OF CONTROL DRINK

Patient Designation	*Pre-Drink 0-Hrs.*	*Hours after Ingestion of Control Drink*		
		2	*4*	*6*
1. Pat.	16.8	13.8	3.9	16.7
2. Hun.	8.3	11.0	7.1	—
3. LaP.	23.4	20.1	19.8	6.0
4. McI.	19.7	19.3	17.6	25.3
5. Ban.	19.8	25.8	14.5	13.2
6. Joh.	21.4	20.9	12.1	7.6
7. Boy.	15.6	20.3	7.5	20.1
8. Cas.	22.4	22.8	21.2	18.1
9. McC.	25.3	22.3	14.3	20.2
10. Gri.	15.7	18.6	22.0	28.0
Means	18.8	19.5	14.0	17.2

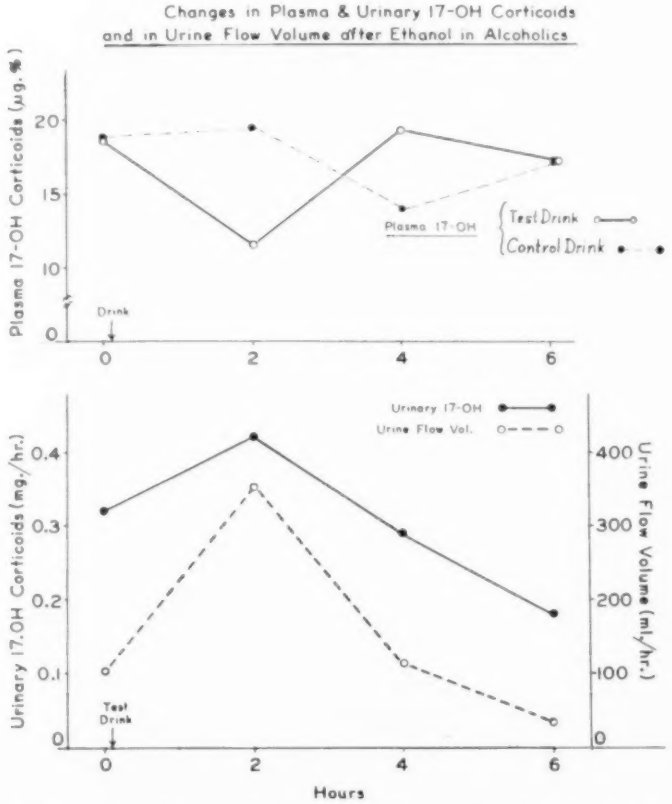

Fig. 3.—Serial changes over 6 hours in urinary 17-hydroxycorticoid and urine flow rates after ethanol Test Drink, with corresponding changes in plasma 17-hydroxycorticoid levels after both Test and Control Drinks.

compounds, secondary to diuresis. This effect does not, however, apply to the 17-ketosteroids since no increase was found in these constituents after ethanol (Experiment 1). This difference in the effect of diuresis upon excretion of 17-hydroxycorticoids and 17-ketosteroids has been reported by others (Mason and Engstrom[7]).

At the 4-hour interval, in contrast to the rise in plasma corticoids after ethanol, there is no such increase after water-loading, the levels remaining lower than base line through 6 hours. The latter two points of the depressed plasma 17-hydroxycorticoid curve after water loading is apparently due to diurnal variation.

It would appear that the lowering of the plasma 17-hydroxycorticoid values at the 2-hour interval after both ethanol and water loading is related to the increased excretion of corticoids in the urine secondary to diuresis. The

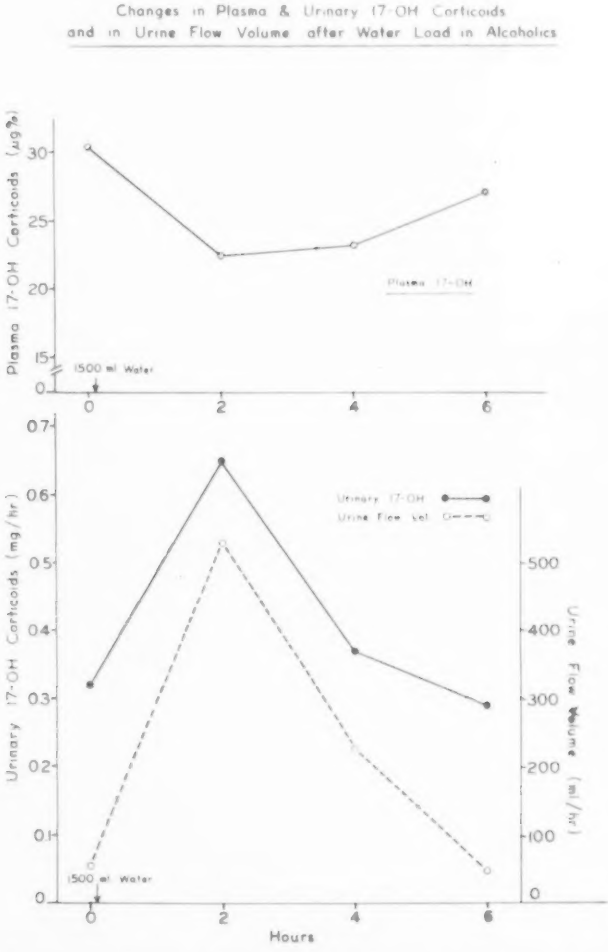

Fig. 4.—Serial changes in plasma and urinary 17-hydroxycorticoids together with urine flow volume in 11 alcoholic patients following ingestion of a 1.5 liter water-load.

TABLE 6.—SERIAL CHANGES IN URINARY AND PLASMA 17-HYDROXYCORTICOIDS IN ALCOHOLICS AFTER A 1500 ML. WATER LOAD

	Baseline (−1 to 0 Hours)			*Hours after Ingestion of Water*								
				0 to 2 Hours			*2 to 4 Hours*			*4 to 6 Hours*		
	Urine		*Plasma*	*Urine*		*Plasma*	*Urine*		*Plasma*	*Urine*		*Plasma*
Patient Designation	*Vol.* (*ml./hr.*)	*17-OH* (*mg./hr.*)	*17-OH* (*μg./100 ml.*)	*Vol.* (*ml./hr.*)	*17-OH* (*mg./hr.*)	*17-OH* (*μg./100 ml.*)	*Vol.* (*ml./hr.*)	*17-OH* (*mg./hr.*)	*17-OH* (*μg./100 ml.*)	*Vol.* (*ml./hr.*)	*17-OH* (*mg./hr.*)	*17-OH* (*μg./100 ml.*)
1. Hu.	78	.72	23.4	550	1.57	—	215	.45	20.4	32	.31	31.6
2. Joh.	24	.04	35.5	410	.29	24.0	365	.28	23.2	3	—	34.0
3. Wal.	47	.38	67.2	830	.88	50.4	97	.05	45.8	7	.06	30.4
4. Pyl.	39	.19	22.6	540	.37	19.0	118	.20	33.8	105	.21	24.0
5. Tes.	58	.54	23.0	402	.48	27.8	217	.26	10.8	41	.29	23.2
6. Goo.	86	.24	48.2	291	.26	19.2	199	.19	23.8	32	.07	41.8
7. Tho.	70	.34	21.4	470	.61	20.3	70	.25	21.8	95	.39	15.3
8. Con.	28	.11	12.2	785	.51	12.0	420	.52	13.7	43	.20	22.3
9. Man.	—	—	34.2	400	.56	18.3	350	.31	26.6	70	.17	22.6
10. Den.	—	—	25.9	445	.97	23.4	195	.98	21.6	60	.30	—
11. Ste.	—	—	21.9	740	.67	9.7	275	.53	15.6	54	.92	28.0
Means	54	.32	30.5	533	.65	22.4	229	.37	23.4	49	.29	27.3

exact nature of this relationship is not immediately apparent. It might be due to increased excretion of hydrocortisone in the urine together with other steroid metabolites, as previously postulated; or it may be due to other as yet undetermined mechanisms. In any case, irrespective of the mechanism, it appears that diuresis is accompanied by a fall in 17-hydroxycorticoids. This relationship has not been previously described.

This effect was of particular interest to us because of the drop at the 2-hour interval in plasma 17-hydroxycorticoids after ethanol where we had anticipated a rise in keeping with the animal experiments previously described (Forbes and Duncan[2], Santrestiban and Swingard[9], Smith[11]). It was our feeling that the diuresis accounted for this apparent paradox. On the other hand, the rise in plasma 17-hydroxycorticoids after ethanol at the 4- and 6-hour intervals, we felt, was due to the actual adrenocortical stimulatory effect of ethanol. In order to separate these effects, we attempted to create a situation where ethanol administration would be accompanied by minimal diuresis. In order to accomplish this we used the well known fact that water loading inhibits the ADH suppressive effect of alcohol (Rosenbaum *et al.*[8]).

(7) *Effect of ethanol with fluid supplementation upon plasma and urinary 17-hydroxycorticoids.* Twelve patients were tested. As in the preceding experiments, the alcohol Test Drink was taken immediately after collection of base line samples. At each hour thereafter, for 4 hours, the patients drank 250 ml. either of water or hypotonic (0.4%) saline. It was found that there was no significant difference in the respective responses of these two groups; therefore we have considered this a single group for the purpose of this experiment.

On examination of the urinary volumes after ethanol, the 12 patients were divided into two groups depending upon the relative degree of diuresis. An arbitrary figure of 350 ml. of urine per hour for the first 2-hour period was chosen as the cut off point, all values lower than this being considered as *minor diuresis*, all values higher as *major diuresis*. The findings are listed in Table 7. In the minor diuresis group there is no significant increase in the mean urinary excretion of 17-hydroxycorticoids at the 2-hour period. On the other hand, all 4 patients showed a marked rise in plasma 17-hydroxycorticoids at the 2-hour interval. In the 8 patients comprising the major diuresis group, there was a rise in the mean urinary 17-hydroxycorticoids. For the blood levels, 4 showed a sharp fall at 2 hours, 3 remained unchanged, and one showed an actual rise. The means for the 2-hour plasma 17-hydroxycorticoids in the minor diuresis group are sharply higher than the resting values; those in the major diuresis group sharply lower. Thus, it appears that when the diuretic effect of ethanol is minimal (namely, less than 350 ml. of urine per hour) there is a minimal urinary corticoid excretion rate with the level of 17-hydroxycorticoids in the plasma showing a rise rather than a fall at the 2-hour interval.

These findings suggest that the rise in plasma 17-hydroxycorticoids between 2 and 4 hours after ethanol, described in Experiment 4, is due to actual stimulation of adrenal cortical activity. Apparently, when for some reason there is no increase in urinary 17-hydroxycorticoids after ethanol, there is a sharp rise in plasma 17-hydroxycorticoids as demonstrated above in the minimal diuresis group. This, it would seem, is probably due to increased production of 17-hydroxycorticoids.

TABLE 7—CHANGES IN PLASMA AND URINARY 17-HYDROXYCORTICOIDS AFTER ETHANOL WITH FLUID SUPPLEMENTATION

	Time after Ethanol Ingestion								
	0 Hours			*2 Hours*			*4 Hours*		
	Urine		*Plasma*	*Urine*		*Plasma*	*Urine*		*Plasma*
Patient Designation	*Vol. (ml./hr.)*	*17-OH (mg./hr.)*	*17-OH (μg./100 ml.)*	*Vol. (ml./hr.)*	*17-OH (mg./hr.)*	*17-OH (μg./100 ml.)*	*Vol. (ml./hr.)*	*17-OH (mg./hr.)*	*17-OH (μg./100 ml.)*
"Minor" Diuresis									
1. Col.	33	.13	10.0	193	.16	25.0	133	.18	14.7
2. Jac.	78	.63	8.9	324	.79	20.0	231	.36	6.2
3. Swe.	35	.13	25.5	305	.29	32.7	51	.02	30.4
4. Fin.	60	.22	3.2	255	.18	8.1	203	.17	13.2
Means:	52	.28	11.9	269	.36	21.5	155	.18	16.1
"Major" Diuresis									
1. Cum.	75	.49	9.8	508	.34	17.5	88	.27	9.7
2. Rod.	155	.89	15.2	398	.45	15.8	130	.26	8.9
3. Hal.	110	.10	16.5	377	.41	6.7	119	.19	22.1
4. Pry.	75	.08	21.9	698	.38	9.2	116	.15	15.3
5. Mos.	190	.46	21.8	603	.46	2.9	115	.21	21.6
6. Jen.	104	.50	19.8	394	.62	11.8	57	.20	10.7
7. Mur.	48	.12	32.0	415	.72	31.0	54	.25	04.7
8. Cog.	55	.25	6.0	375	.80	6.6	73	.19	12.6
Means:	102	.36	17.9	471	.52	12.7	94	.22	14.9

(8) *Changes in eosinophil count 4 hours after ethanol ingestion.* Because of the increased adrenocortical activity suggested by the preceding experiments, a corroborative experiment was done to ascertain whether an eosinopenic response was elicited after the ingestion of ethanol.

Ten alcoholic patients were tested at bed rest and fasting at the same time in the morning on 2 consecutive days, one with the Control Drink and the other with the Alcohol Test Drink. Blood specimens were taken immediately before and 4 hours after the ingestion of the respective drinks.

TABLE 8.—CHANGES IN EOSINOPHIL COUNTS 4 HOURS AFTER ALCOHOL TEST DRINK AND NON-ALCOHOL CONTROL DRINK IN ALCOHOLIC PATIENTS

Patient Designation	*Change Per Cent Eosinophil Count*	
	After Control Drink	*After Test Drink*
1. Via.	−31	−19
2. Cun.	+28	−50
3. O'N.	+19	−54
4. Sul.	− 7	−61
5. Whi.	− 2	−18
6. Fit.	−19	−71
7. Car.	+15	−23
8. For.	− 3	−33
9. Boy.	−14	−42
10. Sch.	− 8	−72

Changes in eosinophil counts are shown in Table 8. Using the criterion of 50% eosinopenia as indicative of adrenocortical stimulation, it is seen that following the Control Drink none of the 10 patients showed this effect (in fact, 3 of them showed eosinophilia), whereas after the alcohol Test Drink, 5 of them did. The ingestion of ethanol is associated with a significant (p less than .05, one-tailed sign test) eosinopenic response.

Discussion. The animal experiments of Smith[11], Forbes and Duncan[2], and Santrestiban and Swingard[9], in which depletion of adrenal cortical ascorbic acid and cholesterol was demonstrated to follow intoxication with ethanol, have generally been accepted to indicate that alcoholic intoxication causes direct adrenal cortical stimulation. Actually, some of our experiments suggest an alternative explanation for these findings in animals. If, as we have indicated, ethanol diuresis causes an outpouring of hydroxycorticoids into the urine, the adrenal cortical depletion might result from a chronically increased loss of hydroxycorticoids.

However, from the results of the first 4 cases in Table 7, illustrated in Fig. 5A, it appears that there is, in addition, a more immediate and direct stimulatory effect of ethanol on adrenal cortical function. The sharp rise in plasma hydroxycorticoids in the absence of the outpouring into the urine, indicates increased hydroxycorticoid production due to adrenal cortical stimulation. Corroborative evidence is provided by the positive effect upon the esosinophil count at 4 hours.

Whether the increased production of 17-hydroxycorticoids be due to stimulation of the anterior pituitary, as postulated by Smith[11], or to some other

mechanism cannot be determined from our data. However, it appears that ethanol does not stimulate 17-ketosteroid production, an effect one would anticipate if the mechanism of the ethanol response were through increased ACTH production. The precise mechanism remains obscure.

Summary. 1. After the ingestion of a single dose of ethanol there is an early diuresis accompanied by increased urinary 17-hydroxycorticoid output and lower plasma 17-hydroxycorticoid levels (at 2 hours). After the initial diuresis, there is a subsequent period characterized by diminished

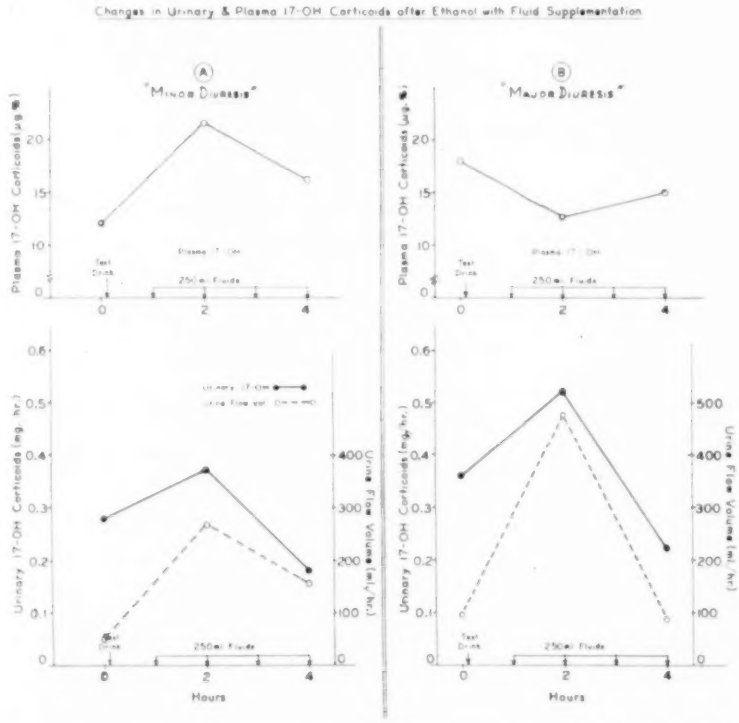

Fig. 5.—Changes in urinary and plasma 17-hydroxycorticoids in alcoholic patients after ingestion of ethanol Test Drink with hourly fluid supplementation.

Perhaps our most significant finding is the close relationship between renal clearance and both plasma and urinary levels of 17-hydroxycorticoids. It would appear from our data that in any reliable study of 17-hydroxycorticoid metabolism, particularly in cases where changes in renal clearance are likely, consideration must be given to both plasma and urinary levels measured concomitantly.

urinary 17-hydroxycorticoids with an increase in plasma 17-hydroxycorticoids to base-line levels (at 4 hours). The urinary 17-hydroxycorticoids remain low for the next 12 hours, returning gradually to base-line values.

2. After diuresis induced by a water-load of 1500 ml., urinary 17-hydroxycorticoids increase and plasma 17-hydroxycorticoids are lowered (at 2 hours), indicating that diuresis is ac-

companied by increased renal clearance and excretion of 17-hydroxycorticoids with a subsequent reduction of plasma 17-hydroxycorticoids.

3. When ethanol is given with fluid supplementation, in some subjects there is a rise in the plasma 17-hydroxycorticoids with no marked changes in the urinary 17-hydroxycorticoids. These findings are suggestive of active adrenal cortical stimulation.

4. After the ingestion of ethanol, there is a significant drop in blood eosinophils at 4 hours. This tends to corroborate the interpretation that ethanol has an active stimulatory effect upon the adrenal cortex.

5. The urinary excretion of 17-ketosteroids, in contradistinction to that of 17-hydroxycorticoids, is not affected by the administration of ethanol or by concomitant changes in renal clearance.

6. The importance of doing concomitant urinary and plasma 17-hydroxycorticoids is indicated, especially when changes in renal clearance are involved.

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SUMMARIO IN INTERLINGUA

Le Acute Effecto Exercite per le Ingestion de Ethanol Super le 17-Hydroxycorticoides del Plasma e del Urina in Subjectos Alcoholic

1. Post le ingestion de un dose unic de ethanol, il occurre precoce diurese accompaniate de un augmento del excretion urinari de 17-hydroxycorticoides e de nivellos reducite de 17-hydroxycorticoides in le plasma. Isto se observa al fin del prime 2 horas. Post le diurese initial, le periodo subsequente es characterisate per un reduction del 17-hydroxycorticoides urinari e per le augmento del 17-hydroxycorticoides del plasma usque al valores de controlo. Iste se observa al fin del prime 4 horas. Le 17-hydroxycorticoides del urina remane basse durante le sequente 12 horas e postea retorna gradualmente al valores de controlo.

2. Post diurese inducite per cargation de aqua (1500 ml), le 17-hydroxycorticoides del urina se augmenta, e illos del plasma se reduce. Iste constatation, que se observa al fin del prime 2 horas, indica que le diurese es accompaniate de un augmento del clearance e del excretion renal de 17-hydroxycorticoides, con un reduction subsequente del 17-hydroxycorticoides del plasma.

3. Quando ethanol es administrate con supplementos de liquido, il occurre in certe subjectos un augmento del 17-hydroxycorticoides del plasma sin marcate

alteration del 17-hydroxycorticoides del urina. Iste constatation pare reflecter un active stimulation del cortice adrenal.

4. Post le ingestion de ethanol, il occurre al fin de 4 horas un significative reduction del eosinophilos del sanguine. Isto pare corroborar le interpretation que ethanol exerce un active effecto stimulatori super le cortice adrenal.

5. Le excretion urinari de 17-cetosteroides, per contrasto con illo del 17-hydroxycorticoides, non es afficite per le administration de ethanol o le concomitante alterationes del clearance renal.

6. Le importantia de studios concomitante del 17-hydroxycorticoides in le urina e le plasma es considerabile, specialmente in casos in que il se tracta de alterationes del clearance renal.

SUMMARIO IN INTERLINGUA

(See page 713 for original article)

Calculos Biliari e Pregnantia: Le Composition de Bile de Vesica Biliari in Gravidas a Termino

Specimens de bile aspirate ab le vesica biliari de 28 gravidas in section cesaree, comparate con specimens aspirate ab le vesicas biliari de 10 femininas normal, resultava in nulle constatation suggestive de alterationes del constituentes del bile intravesical o de alterationes in le function del vesica biliari durante le pregnantia de un genere capace a promover le formation de calculos biliari.

Specimens de bile obtenite per un tubo in forme de T ab un femina de 27 annos de etate in le curso de su pregnantia resultava similemente in un analyse sin ulle indicio de alterationes que poterea ducer al formation de calculos.

A SINGLE DETERMINATION ASSAY OF THROMBOPLASTIN GENERATION AS A TEST OF LIVER FUNCTION*

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A previous study noted that an abnormal thromboplastin generation test is obtained with serum from the majority of patients with documented liver disease (Rabiner and Spaet[5]). Since this defect is frequently demonstrable with serum from subjects who, despite normal standard liver function tests, are suspected clinically of having hepatocellular disease, the serum thromboplastin generation test (S.T.G.T.) can serve as a sensitive index of liver function. This abnormality, also present in patients receiving coumarin drugs, can be duplicated experimentally by incubating serum from a normal individual 24 to 72 hours at 38° C. The coagulation defect responsible for abnormal S.T.G.T. in patients with liver disease, or receiving coumarin drugs, was felt to be caused by depression of factor X (Duckert *et al.*[4]). At the present time, although most investigators believe that factor X is synonymous with Stuart-Prower factor, some still hold that factor X is a separate factor necessary for normal thromboplastin generation (Spaet and Kropatkin[6]).

In patients with viral hepatitis the abnormal S.T.G.T. appears early in the course of the disease, and persists during the period of recovery after standard liver function tests have returned to normal. This suggests that the S.T.G.T. may be sensitive enough to be useful as a screening test for blood donors. To serve such a purpose, however, a liver function test must not only be extremely sensitive, but rapid and simple. Unfortunately the standard S.T.G.T. is time-consuming, requiring approximately 15 minutes for one determination. The purpose of this study is to evaluate a rapid single-determination thromboplastin generation assay as a test of liver function.

Methods and Materials. The subjects studied were normal volunteers, and patients either hospitalized, or attending The Liver Disease Clinic at The New York Hospital-Cornell Medical Center. For the purpose of this study, they are divided into 3 groups on the basis of history, physical examination, and laboratory findings.

Group I: Subjects with no known liver disease, including normal volunteers and patients.

Group II: Patients felt to have clinical liver disease because of either hepatic enlargement or recent hepatitis but whose standard liver function tests are normal.

Group III: Patients with documented liver disease when one or more liver function tests have confirmed the presence of hepatocellular damage.

The tests of liver function employed in this study include the following: serum bilirubin, total protein, albumin, globulin, glutamic oxaloacetic transaminase (S.G.O.T.), alkaline phosphatase, cephalin flocculation, thymol turbidity, and the excretion of sulfobromphthalein. Usually all, and at least two of the above studies, were performed on patients in Groups II and III. Many individuals in

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Group I, the control group, were not evaluated with standard liver function tests.

The following are reagents for the single-determination thromboplastin generation assay. Serum reagent is prepared from 1 to 2 ml. whole blood, agitated slowly with glass beads in a test tube until coagulation occurs, and then incubated at 7° C. for 3 hours. Serum, separated by centrifugation, is diluted 1:20 with 0.5% sodium chloride in imidazole buffer pH 7.35 and then reincubated for exactly one hour prior to use. An "aged" serum is prepared in advance by permitting undiluted serum, collected in the same manner from a normal subject, to incubate at 38° C. for 24 to 72 hours. The standard thromboplastin generation test with this serum, in an otherwise normal system, should be grossly abnormal, with a substrate clotting time of 20 to 30 seconds.

Tricalcium phosphate, or barium sulfate adsorbed oxalated bovine plasma diluted 1:10 with normal saline, is used as plasma reagent. Oxalated bovine plasma is employed as substrate and brain "cephalin" prepared by the method of Bell and Alton[2] is used as a substitute for platelet thromboplastic factor. Imidazole buffer is prepared by adding 15 ml. of 0.2 molar imidazole* to 25 ml. 0.1 normal hydrochloric acid. The pH was then adjusted to 7.35 with additional 0.1 normal hydrochloric acid. Sodium chloride, 0.5 gm., is added to 100 ml. of buffer for this determination.

As a test of liver function increased sensitivity is obtained by assaying the property of small amounts of test serum to correct the defect of aged normal serum rather than by performing a thromboplastin generation test. The single-determination thromboplastin generation assay of "factor X" activity is performed as follows. Before starting, 5 substrate tubes containing 0.1 ml. of oxalated bovine plasma are placed in a water bath at 38° C. A series of 5 generation tubes is then prepared in the same water bath (Table 1). Into each tube is first pipetted 0.1 ml. of a 1:5 dilution of aged serum from a normal subject. One-tenth milliliter of a 1:20 dilution of fresh normal serum is then added to the first tube to serve as a control. Into the 4 remaining test tubes are added 0.1 ml. of a 1:20 dilution of sera to be tested. A different aliquot of diluted serum is added to each test tube so that 4 assays can be performed simultaneously. Four-tenths milliliter of a mixture of equal parts of plasma and platelet reagents (1:10 dilution in saline of adsorbed bovine plasma plus brain "cephalin") are then added to the first test tube and a stopwatch started simultaneously. Forty-five seconds later 0.2 ml. of 0.025 molar calcium chloride (calcium) is added to the first test tube. At 1 minute (15 seconds later) 0.4 ml. of the mixture of plasma and platelet reagents is added to the second test tube to which 0.2 ml. of calcium is added 45 seconds later. The remaining 3 test tubes are treated in a similar manner so that calcium is added to the last tube at 4 minutes 45 seconds. At 5 minutes 45 seconds the first substrate tube is recalcified with 0.1 ml. of calcium. At 6 minutes an 0.1 ml. sample, obtained from the first generation tube, is added to the first substrate tube and a second stopwatch is simultaneously started. The clotting time of the first substrate plasma is noted and recorded. At 6 minutes 45 seconds the second substrate plasma is recalcified, and at 7 minutes an 0.1 ml. sample from the second generation tube is added and the substrate clotting time determined. The remaining 3 generation and substrate tubes are tested in a similar manner, the last determination performed at 10 minutes. For the results to be significant the substrate clotting time of the control mixture should be no longer than 14 seconds. If the control is over 14 seconds, a new normal or aged serum specimen should be obtained and the run repeated. The difference in seconds between the control system and each test system is then determined. It is extremely important to prepare sera exactly as indicated; that is, whole blood agitated slowly in glass beads, permitted to incubate for 3 hours on the clot, diluted in 0.5 gm.% sodium chloride in imidazole buffer pH 7.35, and finally reincubated for exactly one hour after dilution in the saline buffer solution. Other buffers and diluting solutions have been tried with unsatisfactory results.

Results. The results of the single-determination thromboplastin generation assay are summarized in Fig. 1. Noted are deviations from normal of assays with each test serum. For example, if the substrate clotting time of the control is 10 seconds, and the test system 14 seconds, a plus 4 is indicated. However, a minus 2 is recorded if, with the same control, an 8-second time is obtained with the test mixture.

*Obtained from Edcan Company, South Norwalk, Conn.

A plus 2 or less was obtained with all sera from subjects except one with no known liver disease (98%). On the other hand, 42 of 45 (93%) sera from patients with liver disease were above normal range, while 17 of 27 (63%) sera of the group with suspected liver disease but normal standard liver function tests were abnormal.

Several subjects had 2 or more assays of thromboplastin generation by the single-determination method. The results generally mirrored the patient's clinical course. Discrepancies were

TABLE 1.—THE SINGLE DETERMINATION THROMBOPLASTIN GENERATION ASSAY

	Test Tube Number				
Time	*1*	*2*	*3*	*4*	*5*
0	0.1 ml.–1:5 dilution "aged" normal serum.	0.1 ml.–1:5 dilution "aged" normal serum.	0.1 ml.–1:5 dilution "aged" normal serum.	0.1 ml.–1:5 dilution "aged" normal serum.	0.1 ml.–1:5 dilution "aged" normal serum.
0	0.1 ml.–1:20 dilution fresh normal serum.	0.1 ml.–1:20 dilution test serum No. 1.	0.1 ml.–1:20 dilution test serum No. 2.	0.1 ml.–1:20 dilution test serum No. 3.	0.1 ml.–1:20 dilution test serum No. 4.
Start stop-watch I.	0.4 ml. mixture equal parts plasma & platelet reagents.	—	—	—	—
0 mins. 45 secs.	0.2 ml. 0.025 M. $CaCl_2$	—	—	—	—
1 min. 0 secs.	—	0.4 ml. mixture equal parts plasma & platelet reagents.	—	—	—
1 min. 45 secs.	—	0.2 ml. 0.025 M. $CaCl_2$	—	—	—
2 mins. 0 secs. to 4 mins. 45 secs.	—	—	Same as Tubes 1 & 2.	Same as Tubes 1 & 2.	Same as Tubes 1 & 2.
6 mins.	Remove 0.1 ml. sample—add to recalcified substrate & time with S.W. II.	—	—	—	—
7 mins.	—	Remove 0.1 ml. sample—add to recalcified substrate & time with S. W. II.	—	—	—
8 mins. to 10 mins.	—	—	Same as Tubes 1 & 2.	Same as Tubes 1 & 2.	Same as Tubes 1 & 2.

noted in patients receiving multiple transfusions, where the test varied depending on the length of time since the last transfusion and probably the age of the administered blood. One patient with known cirrhosis of the liver was studied while receiving multiple transfusions for gastrointestinal hemorrhage complicating a gastric ulcer. The thromboplastin generation assay initially was plus 1. When transfusions were discontinued, repeat assays of plus 4, and, a week later, plus 12 were obtained. In Fig. 1 all results obtained from this patient are included in the group of subjects with liver disease and abnormal liver function tests.

Another patient with cirrhosis was admitted with delirium tremens and a ruptured appendix (Fig. 2). His initial liver function tests were grossly abnormal, as was the single-determination thromboplastin generation assay. An appendectomy was performed and the patient treated with antibiotics, fluids, and other supportive measures. Despite a stormy postoperative period, his condition gradually improved and he was discharged after 7 weeks in the hospital. Both standard liver function tests

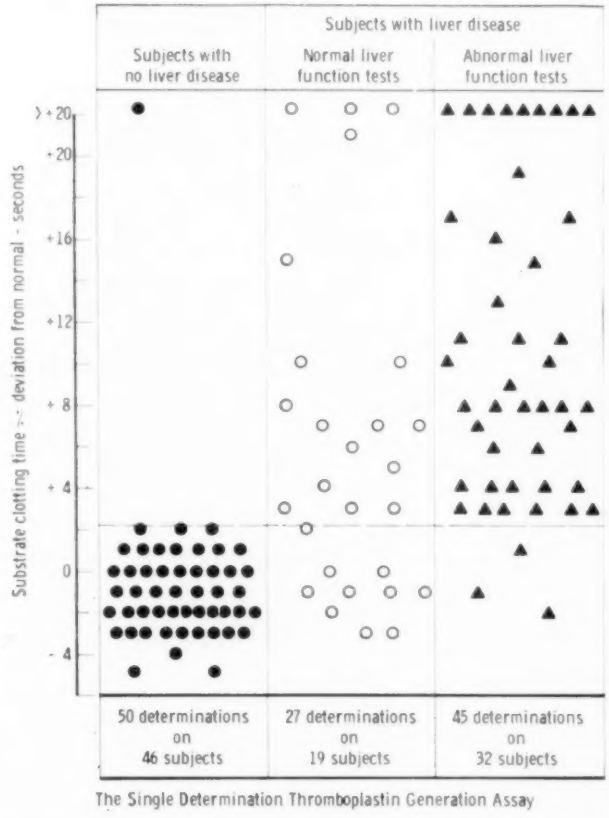

Fig. 1.—Results with the single-determination thromboplastin generation assay in subjects tested.

and single-determination thromboplastin generation assay returned almost to normal.

Discussion. The single-determination thromboplastin generation assay, as described in this study, is not designed to test all factors in serum necessary for generation of intrinsic thromboplastin, but specifically coagulation factor or factors diminished or lacking in aged normal serum. For this reason deficiencies in P.T.C. (Aggeler *et al.*[1]), (Christmas Disease [Biggs *et al.*[3]]), will not alter the test, since this factor is supplied by aged serum added to each tube. The nature of the coagulation defect in aged serum is controversial and presently under study in this laboratory. As previously noted, the relationship between factor X and Stuart-Prower factor has not been established to the satisfaction of all workers interested in this aspect of blood coagulation. This problem is not an issue in the present study, which is rather concerned with utilizing, as a test of liver function, the high incidence of this abnormality in patients with hepatocellular disease.

The data indicate that the single-determination thromboplastin generation assay can be employed as a sensitive liver function test. While 3 of the 45 determinations on patients with known liver disease were normal, poor generations were obtained with 17 of 27 sera from subjects who, despite normal standard liver function tests, had clinical evidence of hepatic disease. Furthermore, none of the standard liver function tests, considered individually, was as sensitive as the single-determination thromboplastin generation assay. The one apparent false positive is noted and may possibly represent a case of subclinical hepatitis.

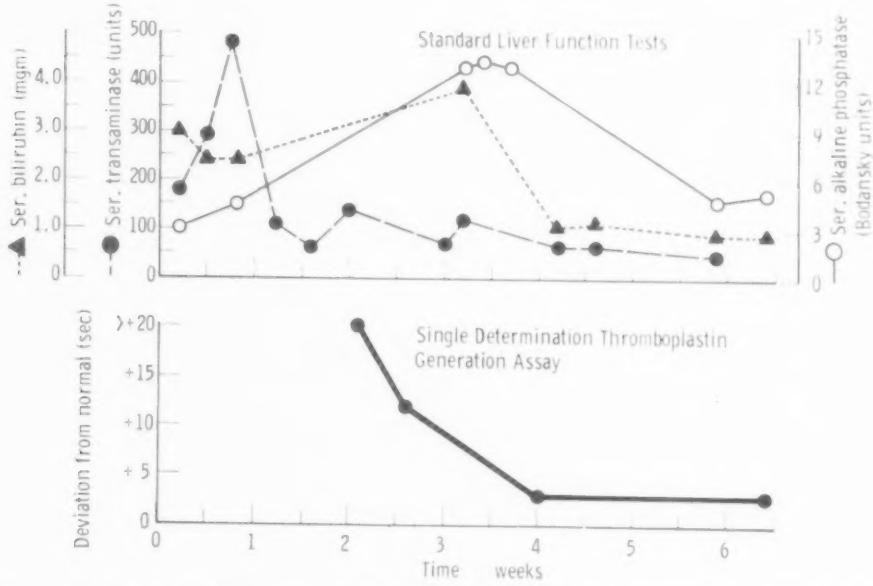

Fig. 2.—Results of standard liver function tests and the single determination thromboplastin generation assay in a patient with cirrhosis, delirium tremens, and ruptured appendix.

Abnormal assay in the absence of liver disease may be encountered with sera from patients receiving coumarin drugs or from those with congenital abnormalities of the factor under study. The latter is extremely rare, however, and subjects receiving coumarin drugs were not included in this study. Since the coagulation defect can be corrected by the administration of fresh plasma, normal assays may be obtained in the presence of hepatic disease in patients who have been recently transfused with fresh blood or plasma.

The test is influenced not only by the magnitude of the coagulation defect of patients' serum, but by the aged normal serum as well. The latter must be carefully controlled to arrive at consistent results. In an otherwise normal system, a standard thromboplastin generation test with aged serum should yield a substrate clotting time, after 6 minutes incubation, of greater than 25 seconds. On the other hand, multiple defects appear in serum aged for extended periods of time. For this reason, when the control system of the single-determination thromboplastin generation assay is over 14 seconds, a different aged serum should be chosen. It is usually advisable to store samples of normal serum periodically so that sera at various stages of aging are always available. Once the desired defect is approached, the serum can be transferred from the 38° C. water bath and stored in a refrigerator at 4 to 5° C. The desired abnormality will appear when serum is aged at room temperature or 4 to 5° C.; however this will occur more slowly.

The single-determination thromboplastin generation assay can be run in any laboratory equipped to perform standard thromboplastin generations or even prothrombin times. The initial incubation period of 4 hours is ample time to test the aged serum and to prepare the other reagents used in the test. Once everything is ready, 4 assays can be determined in approximately 12 minutes. All necessary reagents for the test are either available commercially or prepared with relative ease in the laboratory.

Further work is necessary to determine exactly how sensitive the single-determination thromboplastin generation assay is, and how it is influenced by various types of liver disease. The data obtained so far suggest that it is abnormal in both chronic hepatic disease such as Laennëc's cirrhosis and very early in acute viral hepatitis. At present its use as a screening test for blood donors is being evaluated.

Summary and Conclusions. 1. A method for performing a single-determination thromboplastin generation assay is described. It is designed, in this instance, to test the ability of sera to correct the first-phase coagulation defect of aged serum.

2. Ninety-three per cent of sera from patients with established liver disease were abnormal when tested with the single-determination thromboplastin generation assay. Sixty-three per cent of sera from subjects with clinical evidence of liver disease were abnormal despite normal standard liver function tests. There was one apparent false positive out of 50 controls with no demonstrable liver disease.

3. An abnormal test can be obtained in the absence of liver disease with sera from patients receiving coumarin drugs or with a congenital deficiency of the factor under study. A normal test in the presence of liver disease may be encountered with sera from patients receiving transfusions of fresh blood or plasma.

4. The single-determination thromboplastin generation assay is apparently a sensitive, practical, liver function test.

It is abnormal in chronic hepatic disease as well as early in acute viral hepatitis. The reagents necessary to perform the test are easily obtained and it can be run in every laboratory equipped to do prothrombin times.

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SUMMARIO IN INTERLINGUA

Proba del Generation de Thromboplastina a Determination Singule Como Test del Function Hepatic

Es describite un methodo pro essayar le generation de thromboplastina per medio de un sol determination. In le presente situation, illo es destinate a evalutar le capacitate de seros de corriger le defecto coagulatori del prime phase in sero de etate.

Novanta-tres pro cento de seros ab patientes con establite morbo hepatic manifestava anormalitates in iste test del generation de thromboplastina. Sexanta-tres pro cento del seros esseva anormal in casos in que le patientes exhibiva signos clinic de morbo hepatic durante que le tests standard de function hepatic esseva normal. Le studio de 50 subjectos de controlo sin demonstrabile morbo hepatic resultava in un caso de positivitate apparentemente false.

Un reaction anormal pote esser causate in le absentia de morbo hepatic per therapia a coumarina o per congenite carentia del factor sub investigation in le sero del donator. Un reaction normal in le presentia de morbo hepatic pote esser incontrate in le caso de seros ab patientes que recipe transfusiones de plasma o sanguine fresc.

Le essayo del generation de thromboplastina a determination unic es apparentemente un sensitive e practic test del function hepatic . Illo es anormal in chronic morbo hepatic si ben como al initio de acute hepatitis virusal. Le reagentes requirite per le essayo es facile a procurar. Le essayo pote esser effectuate in omne laboratorio equipate a determinar tempores de prothrombina.

GALLSTONES AND PREGNANCY: THE COMPOSITION OF GALLBLADDER BILE IN THE PREGNANT WOMAN AT TERM*

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Despite the commonly held belief that the formation of gallstones is somehow related to pregnancy, a recent clinical and cholecystographic study of pregnant women in Detroit yielded little if any evidence to support such a view (Large, Lofstrom and Stevenson[9]). Should there indeed be any relationship between pregnancy and gallstone formation, the composition of gallbladder bile in the pregnant woman at term ought to vary considerably from the normal. Years ago Riegel and associates[15] studied gallbladder bile obtained from 34 pregnant women at the time of Cesarean section and compared the findings with those obtained from the normal gallbladder bile of 2 patients. They reported increased cholesterol concentration and decreased bile salt concentration in their pregnant patients and thought that these findings predisposed toward gallstone formation. At about the same time Potter[12] analyzed samples of bile aspirated from the gallbladders of 390 pregnant women at Cesarean section; and he too concluded that the bile obtained from pregnant women was abnormal, although he was unable to reach any conclusions regarding the relationship of pregnancy to gallstone formation.

Since our clinical survey of pregnant women failed to reveal a direct relationship between pregnancy and gallstone formation; and since the reports of Riegel *et al.*[15] and of Potter[12] suggested such striking changes in gallbladder bile, it seemed to us that further study of gallbladder bile of pregnant women at term might be illuminating. Accordingly, bile aspirated from the gallbladders of a number of pregnant women at Cesarean section was studied and compared with samples obtained from normal gallbladders during operations for other unrelated conditions. Bile aspirated from the gallbladders of several patients with gallstones also was analyzed and the results of the studies in these 3 groups form the basis for this report. There is also included the case report of a woman who had T-tube drainage of her common duct throughout pregnancy, and bile samples analyzed at appropriate intervals. We think these latter data are important because, although Potter and others have thought

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that gallbladder function changes during pregnancy, there is also the possibility that the endocrine changes of pregnancy might affect liver function so as to change the composition of hepatic bile itself, and thus also to change the composition of gallbladder bile.

Method. Bile was aspirated with a fine gauge needle and syringe from the gallbladders of 28 unselected pregnant women at the time of Cesarean section. Although no attempt was made to close the needle opening in the gallbladder, the only symptom that developed in these patients was occasional transient upper abdominal pain which probably indicated the leakage of a small amount of bile. Bile aspirated from the gallbladders of 8 patients who had no evidence of gallbladder disease, and who were not pregnant, constituted the control group. These latter samples of bile were obtained during operations on the upper abdomen for conditions other than biliary tract disease. After the bile was aspirated in this second group, a single fine suture was used to close the needle opening in the gallbladder, care being taken not to allow the suture to penetrate the gallbladder mucosa. In the third group of cases (those with stones) the bile was aspirated from the gallbladder at the time of cholecystectomy before the gallbladder itself was opened. In all instances the bile was either analyzed within a short time following its aspiration or else it was freeze-dried and kept in the frozen state until the determinations were performed. The actual methods of analysis were as follows:

CHOLESTEROL. The total cholesterol was determined by a modification of the Liebermann-Burchard (Abell *et al.*[1]) reaction after preliminary extraction of cholesterol from the bile.

CHLORIDES. These were determined by the method of Schales and Schales[16] performed on the Somogyi filtrate.

CHOLIC ACID. Was performed by the method of Irvin, Johnston and Kopala[4].

THE TOTAL BILE ACIDS were determined by a photofluorometric method worked out by Levin, Johnston and Irvin[10].

SODIUM, POTASSIUM AND CALCIUM were determined by a flame photometer described by Overman and Davis[11].

THE PHOSPHOLIPIDS were determined by the method of Youngburg and Youngburg[17], and Fiske and Subbarow[3]. By the use of these methods recovery of the various constituents was possible to within 5 to 10% and aliquot determinations gave checks sufficiently accurate for our purpose.

Results. Some of the specimens drawn early in the study of pregnant women had only chloride determinations performed upon them, because this had been thought to be the most significant finding in previously reported work, but in the more recently obtained samples, complete chemical profiles were done, so that in 12 of the 28 pregnant cases fairly complete data are available, as shown in Table 1. These results are tabulated in increasing concentration of chloride. Table 2 gives the control values tabulated in a similar manner, with the 8 cases again arranged in increasing chloride concentration; and finally in Table 3 are shown the analyses of bile from the patients with gallstones. Table 4 gives the minimum, maximum and average values for each of the 3 groups.

The following case report is that of a patient who had bile specimens drawn from the common duct via T-tube at appropriate intervals during pregnancy and the various components analyzed as shown in Figs. 2 and 3.

Case Report. G.W., a gravida 5, para 4, 27-year-old colored woman was admitted to the Detroit Receiving Hospital January 31, 1957, and discharged February 26, 1957. Her gallbladder had been removed at another hospital 18 months previously, and she now complained of right upper abdominal pain and vomiting. An intravenous cholangiogram showed a stone in the common duct. On February 13, 1947, the common duct was explored and a stone removed. The duct was drained by means of a T-tube. Her last menstrual period had occurred about January 12, 1957, and a pregnancy test was positive.

The patient agreed to have her T-tube left in place during the pregnancy, and bile samples were drawn frequently for analysis.

During July and August, 1957, she was treated for cystitis and pyelitis; and although her expected date of confinement for the

TABLE 1.—GALLBLADDER BILE. PREGNANT PATIENTS AT CESAREAN SECTION

No.	Date	Chloride mEq./L.	Cholesterol mg./100 ml.	Phospholipids mg./ml.	Cholate mg./ml.	Total Base mEq./L. Na	K	Ca
(1)	3-14-56	7			52.0			
(2)	5- 4-56	8.2			50.5			
(3)	2-24-56	9.6			63.5			
(4)	3-20-56	9.6						
(5)	3- 8-56	10.3						
(6)	12-13-57	12.0	774	32.8	59.7	260	15.0	15.2
(7)	3-14-56	14.4			49.0			
(8)	12- 3-56	18.0	767	40.5		256	17.5	31.4
(9)	11- 9-55	23.0						
(10)	12-20-57	24.5	657	54.7	48.7	300	10.0	11.9
(11)	11-11-55	25.8						
(12)	3-26-56	26.0			52.0			
(13)	3-23-56	26.8			27.0			
(14)	3-27-58	28.0	695	21.6	58.2	236	14.2	25.6
(15)	3- 2-56	30.0			51.4			
(16)	5- 3-56	41.0			29.5			
(17)	1-31-58	42.0	800	21.33	32.3	200	14.0	8.6
(18)	11-20-57	43.0	655	29.9	17.8	211	16.0	17.0
(19)	M.J.	45.4						
(20)	11-10-55	46.0						
(21)	5- 4-56	54.7			16.0			
(22)	12-11-56	67	225	10.4		150	15.0	8.7
(23)	12-16-57	68	430	28.5	22.4	170	10.0	7.2
(24	1-24-58	68	529	12.13	20.4	180	8.4	3.9
(25)	3-27-58	87.5	370	8.55	22.4	200	9.4	12.6
(26)	3-11-58	90	209	9.95		146	8.9	9.4
(27)	W.W.	103.7						
(28)	12- 5-57	110.0	471	10.1	26.1	360	24.0	33.2

TABLE 2.—NORMAL GALLBLADDER BILE

Case No. Date	Chloride mEq/L.	Cholesterol mg./100 ml.	Phospholipid mg./ml.	Cholate mg./ml.	Total Base mEq./L. Na	K	Ca
(1) 10-18-57	18	635	40.9	64.0	240	16.7	30.0
(2) L.J. 7-30-57	32	387	38.6	32.2	229	17.6	26.6
(3) K.E. 6- 3-57	35	507	38.1	29.2	236	13.1	24.5
(4) E.C. 6-17-57	43	517	29.3	50.5	100	7.0	9.3
(5) M.G. 10-21-57	46	692	25.3	47.6	231	14.3	19.4
(6) J.S. 4-29-57	56	568	22.4	16.0	206	14.0	23.3
(7) D.A. 7-22-57	62	530	25.9	17.5	192	12.5	19.4
(8) N.I. 9-12-56	83.5	184	10.5	18.3	171	6.3	8.0

TABLE 3.—GALLBLADDER BILE—GALLSTONE CASES

Case No. Date	*Chloride mEq/L.*	*Cholesterol mg./100 ml.*	*Phospholipid mg./ml.*	*Cholate mg./ml.*	*Total Base mEq./L.*		
					Na	*K*	*Ca*
(1) O.S. 2-25-59	32	1031	48.4	27.0	126	11.0	9.2
(2) J.W. 1-14-59	48	1080	37.8	36.3	234	11.6	20.2
(3) A.M. 4- 6-59	73	567	31.0	40.0	219	14.5	15.2
(4) D.B. 5- 1-59	92	124	7.9	8.7	149	8.7	9.4
(5) K.B. 2- 2-59	121	48.4	1.93	1.3	134	4.1	3.8
(6) P.L. 3-19-59	130	281	12.0	10.9	168	6.4	7.0
(7) F.W. 5- 4-59	140	97.8	0.85	1.3	119	2.9	3.6
(8) J.M. 3-19-59	160	271	11.2	6.5	216	15.6	23.4

TABLE 4.

Gall Bladder Bile	*Values*	*Chloride mEq./L.*	*Cholesterol mg./100 ml.*	*Phospholipids mg./ml.*	*Cholate mg./ml.*	*Total Base mEq./L.*		
						Na	*K*	*Ca*
Pregnancy Cases at Cesarean Section	Minimum	7	209	10.1	16	146	8.4	3.9
	Maximum	110	800	40.5	63.5	360	17.5	33.2
	Average	31	548	23.4	39	220	13.5	15.4
Normal Gall-Bladder Bile	Minimum	18	184	10.5	16	100	6.3	8.0
	Maximum	83.5	692	43.9	64	240	16.7	43.0
	Average	47	502	29	34	200	12.7	20.0
Gallstone Cases	Minimum	32	48.4	0.85	1.3	119	2.9	3.6
	Maximum	160	1080	48.4	40	234	14.5	23.4
	Average	100	450	18.9	16.5	170	9.3	11.5

pregnancy was September 17, 1957, she entered Herman Kiefer Hospital August 25, 1957, and delivered a 6 lb. 2 oz. fresh stillborn male infant shortly thereafter.

The changes in the blood and bile that occurred during the pregnancy are shown in the accompanying charts (Figs. 1, 2 and 3).

damage to the gallbladder wall occurs, the decrease in chloride concentration is not noted and the chloride concentration may then approximate that of hepatic bile. It was therefore thought convenient to tabulate our results on

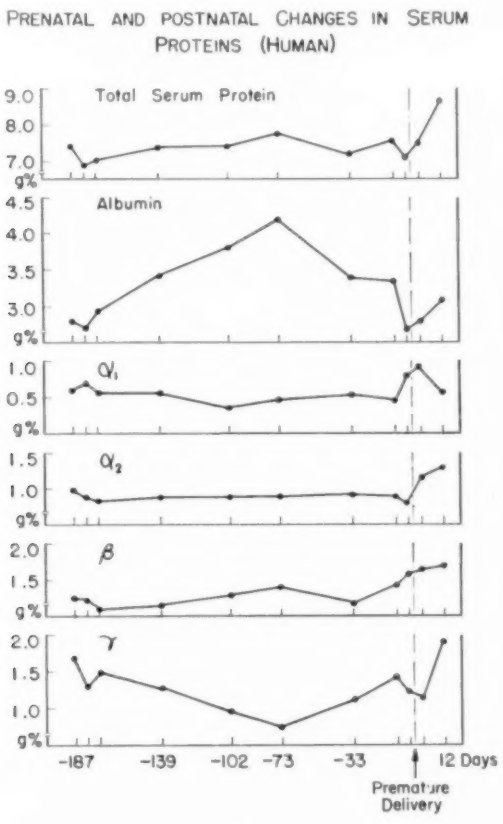

Fig. 1

Discussion. In 1932, Ravdin, Johnston *et al.*[13] showed both experimentally and clinically that the earliest chemical change in gallbladder bile occurring with damage to the gallbladder wall is an increase in chloride concentration. Bile subjected to normal gallbladder activity decreases in chloride concentration to low levels but as the basis of chloride concentration as an indicator of possible gallbladder damage.

The tables show marked variation in all constituents of the bile in both pregnant and normal individuals. It occurred to us that this marked variation might be associated with the different degrees of concentration of the

bile in the gallbladder; if so, bile pigment determinations ought to represent a fairly accurate index of the degree of concentration. Accordingly, in a small number of cases, including both pregnant and nonpregnant women, determinations of the bile pigment concentration were performed (Janowar[6]) and the degree of pigment concentration used as a common denominator to reduce all the other chemical determinations to an equivalent value and in this way to eliminate as much as possible the factor of dilution or concentration of bile. Unfortunately, examination of these figures did not reveal any closer correlation of the various determinations in either the pregnant or normal group than had existed prior to the introduction of the concentration factor; therefore, these figures were discarded and are not included in the present report.

PRENATAL AND POSTNATAL CHANGES IN BILE PROTEINS AND BILE PIGMENTS (HUMAN FISTULA BILE)

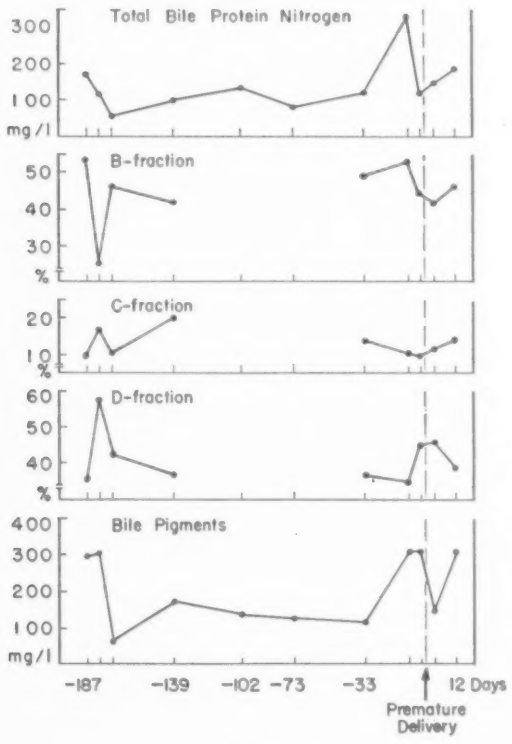

Fig. 2

The variation found in the values of all the constituents of bile does not permit the attachment of any great importance to the individual results. However, we believe the overall findings to be quite significant. As Table 4 shows, not only the chloride determinations but also the cholesterol, total cholate, bile salt and base determina-

tions correspond quite closely in the pregnant and normal groups and do not tend to indicate or suggest anything which might possibly be construed as an alteration toward the precipitation of cholesterol and stone formation. Indeed the average cholesterol concentration of normal gallbladder bile was found only slightly less than was the cholesterol concentration in the pregnant women at term and the cholate determinations were almost identical in the two. It has been generally accepted in the past, and it is our opinion on the basis of recent work as well (Large and Johnston[8]), that the precipitation of cholesterol is the most important single factor in the formation of gallstones; since the degree of saturation of human bile with respect to cholesterol thus assumes equal importance, and since the holding power of cholesterol relates to the bile salts (Andrews, Schoenheimer and Hrdina[2]) and lecithin (Isaksson[5]) within the bile, we believe that these findings are of real importance; and since, as already mentioned, the chlo-

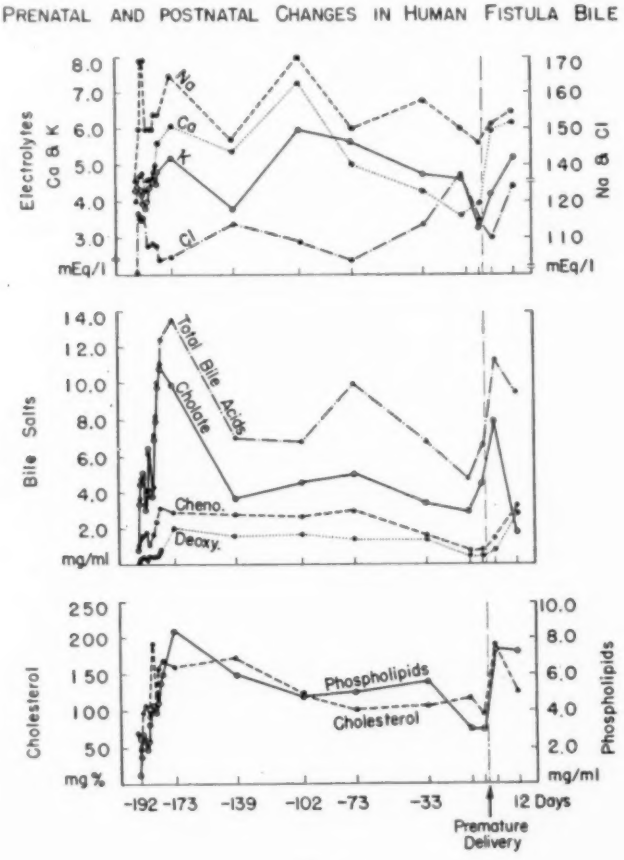

Fig. 3

ride concentration gives a fairly accurate index of damage to the gallbladder wall, it also seems clear to us that this failure of the pregnant woman's gallbladder bile to show an increase in chloride concentration constitutes further evidence that gallbladder function in pregnant women has not changed appreciably from the normal.

The data recorded in Fig. 3 and obtained from samples of hepatic bile drawn via T-tube from the common duct of the patient, G.W., throughout and after pregnancy are interesting and also show marked variation. However, these figures too fail to show any consistent changes in bile salts, phospholipids, or cholesterol itself which might in any way be interpreted as tending toward cholesterol precipitation and stone formation.

Thus neither liver bile nor gallbladder bile appear to be altered appreciably during pregnancy in any way which might induce stone formation.

The results obtained in the patients with gallstones are affected by the fact that some of the specimens were obtained from nonfunctioning gallbladders with very low cholesterol values. However, the findings in general indicate varying degrees of damage to the gallbladder wall and thus to gallbladder function and simply illustrate the direction which one may expect changes in the bile to occur as stones begin to form.

Summary and Conclusions. Bile aspirated from the gallbladders of 28 pregnant women at Cesarean section and compared with the bile aspirated from the gallbladders of 10 normal women yielded no evidence to suggest alteration in the constituents of gallbladder bile or change in the function of the gallbladder during pregnancy which might lead to the formation of gallstones.

Bile samples obtained via T-tube from a 27-year-old woman during the course of her pregnancy likewise failed, when analyzed, to reveal any change which might lead to stone formation.

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(Interlingua on page 705)

MORPHOLOGICAL ASPECTS OF RENAL GLYCOSURIA

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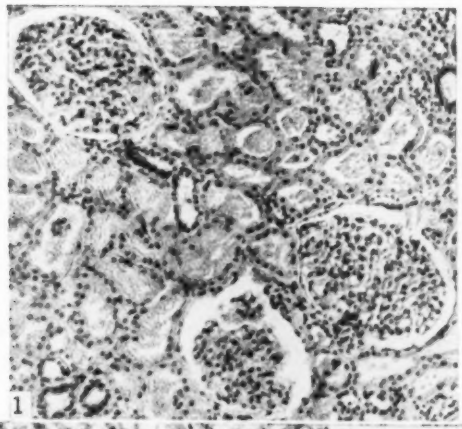
The pathogenesis of renal glycosuria is obscure. Functional studies have failed to provide satisfactory answers to the following questions: 1) Why is proximal tubular reabsorption of glucose defective (Mudge[7], Rhodin[10]), while other transport systems are normal? 2) Is renal glycosuria a true disease or a syndrome with several pathogenetic causes? 3) Is the disorder limited to some nephrons or does it affect all the nephrons uniformly? 4) Are the cells of every proximal tubule uniformly involved? Few attempts have been made to study possible morphologic or enzymatic tubular defects in renal glycosuria, perhaps because of the very benign course of this condition. Monasterio *et al.*[6] using surgical biopsy of the kidney, demonstrated some characteristic "dysplastic and hypoplastic alterations" in the proximal tubules. Similar but less marked alterations were found in 2 other cases of renal glycosuria (Biasotti, Patalano and Mancini[1], Monasterio[5]). On the other hand, a normal histologic appearance was found in a case of normoglycemic glycosuria with pituitary tumor (Grote and Heilmann[2]), in a case of renal glycosuria of type A with essential hypertension (Reubi[8]), and in 2 cases of typical renal glycosuria (Kark[3], Reubi and Cottier[9]). An insignificant decrease in alkaline phosphatase activity was found in one case using histochemical methods (Monasterio[5]), and in one case using microdissection and quantitative dosage (Kark[3]).

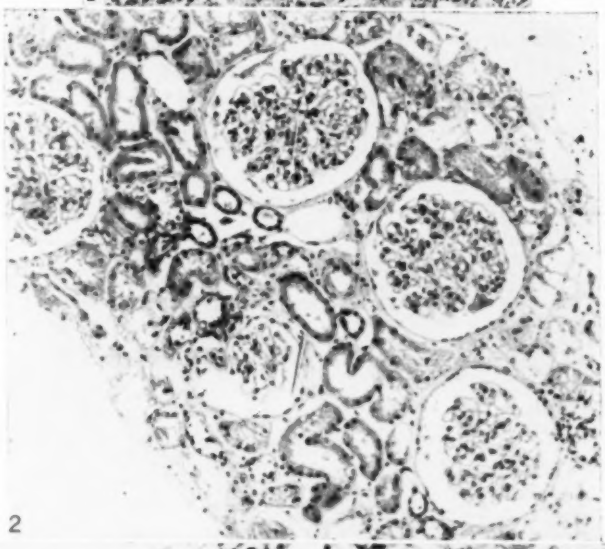
Material and Methods. The renal function of 5 members of 2 different families affected by renal glycosuria was studied. In 4 of them, kidney biopsy was performed with a Turkel or a Silverman-Franklin needle, with the patient in prone position, according to a method previously described (Leonardi and Ruol[4]). Specimens useful for histologic and histochemical examination and for electron microscope studies were obtained in 3 cases. The specimens were fixed in 96% alcohol and stained with hematoxylin-eosin and by the method of Mallory. Each specimen contained more than 10 glomeruli and many proximal and distal tubules. The histochemical distribution of the alkaline phosphatase was determined according to Gomori. A few fragments, about 1 mm. in size, were separated quickly from the biopsy specimen, fixed immediately in 1% osmium tetroxide and prepared for electron microscopic studies.

Results. In all 5 subjects the urinary and blood findings were normal (Table 1). Concentration and dilution tests and renal clearance studies demonstrated excellent renal function. Fasting blood glucose, glucose and insulin tolerance were within normal limits. In 4 patients the threshold for glucose was between 60 and 100 mg. per 100 ml. and glycosuria was continuous; in the fifth case glycosuria was intermittent without any apparent relation to blood glucose concentration. The glucose Tm (tubular excretory mass) was found significantly below normal in 2

TABLE 1.—BLOOD, URINE AND RENAL FUNCTION FINDINGS IN 5 CASES OF RENAL GLYCOSURIA.

Case	Routine Urinalysis	Blood NPN mg./100 ml.	Dilution Test sp. gr.	Conc. Test. sp. gr.	Glomerular Filtration Rate ml./min.	Renal Plasma Flow ml./min.	Glucose Tm gm./min.	Water Tubular Reabsorption %	Blood Glucose mg./100 ml.	Glucose Threshold mg./100 ml.	Glucose Tolerance max. mg./100 ml.	Glucose Tolerance normal within hr.
D.V.V.	normal	20	1001	1035	180	830	250	93.7	110	70	132	2½
D.V.	normal	25	1002	1030					92	10	128	2
S.T.	normal	18	1001	1030	127	412	201	98	110	110	196	2½
S.E.	normal	30	1001	1032	130				110	70	188	1½
S.O.	normal	24	1001	1034	122				102	70	164	1½





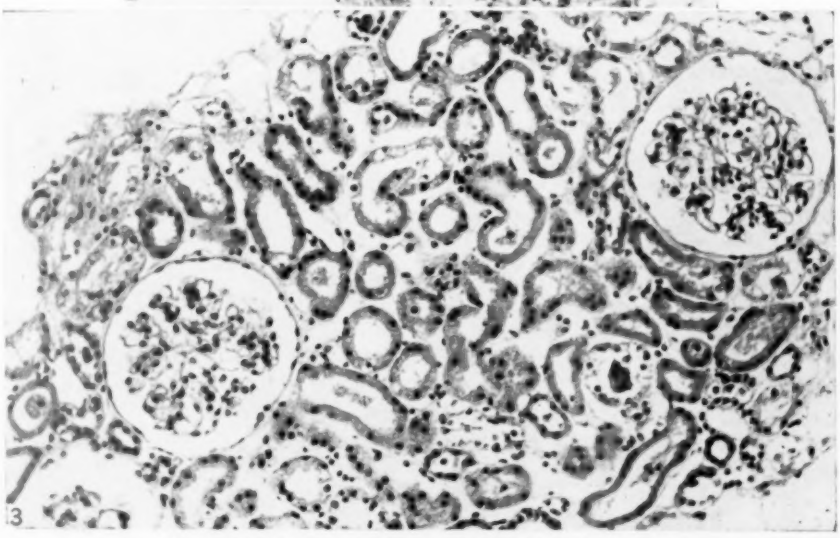


Fig. 1.—Case D.V.V. Renal biopsy. Normal histologic appearance.
Fig. 2.—Case S.E. Renal biopsy. Normal histologic appearance.
Fig. 3.—Case S.O. Renal biopsy. Normal histologic appearance.

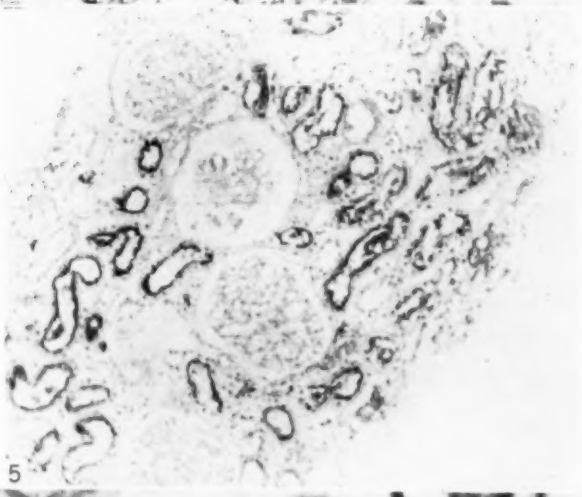
subjects in which it was measured. This observation suggests the existence of a functional defect uniformly distributed throughout the whole population of nephrons.

The histologic appearance of bioptic specimens was identical in all 3 subjects (Fig. 1, 2 and 3). The glomeruli appear normal with a thin basement membrane and normal arterioles. The proximal tubules also appeared normal in volume, thickness and aspect of nuclei, basement membrane and cytoplasm. A few granular deposits in the tubular lumen are a normal finding when the tissue is fixed in alcohol. No alterations were noted in the loops of Henle, the distal tubules, the interstitial tissue or in the blood vessels. Alkaline phosphatase activity could be demonstrated easily in all proximal tubules (Fig. 4 and 5), especially in the brush-border of the cells (Fig. 6). It could be demonstrated also in the endothelial cells of peritubular capillaries and of the arterioles, but not, as a rule, in the glomeruli and distal tubules.

In 2 cases, the study of several proximal tubules by means of the electron microscope did not reveal significant structural alterations which could be considered characteristic of renal glycosuria (Fig. 7 and 8), although the following changes were noted: 1) tubular cells contained a great number of vacuoles, 2) cell membrane was poorly developed and showed widely separated invaginations, 3) some mitochondria were wrinkled and vacuolated and they appeared generally darker, smaller, and shorter than normal, 4) there were longitudinal fissures in the basement membrane, 5) the cell brush-border was normal, some of its microvilli were densely packed, others were short and rather well separated. These changes affecting many different structures are believed to be the result of wrinkling or imbibition, and probably are, at least in part, technical artifacts, similar to those described by others (Sjöstrand[11], Taggart[12]). No other changes were noted with certainty and one is forced to conclude that not even the electron microscope permits detection of alterations of the proximal tubules which could be considered characteristic of renal glycosuria.

COMMENT. Three cases of renal glycosuria with essentially negative histologic findings have been described. These cases resemble some (Kark[3], Reubi[8], Reubi and Cottier[9]), but differ from other cases (Biasotti, Patalano and Mancini[1], Monasterio *et al.* [5,6]) previously described. The presence of a normal alkaline phosphatase activity in the tubular cells agrees with current hypotheses which give little importance to phosphorylation-dephosphorylation reactions for tubular reabsorption of glucose. In 2 typical cases of renal glycosuria the ultramicroscopic structure showed no characteristic changes, although some alterations believed to be technical artifacts were seen. The structure of the brush-border, which is believed to be involved in the enzymatic process of glucose reabsorption, appeared normal. Although no histological or ultramicroscopic alterations are likely to occur in most cases of renal glycosuria, in some cases, such as the one described by Monasterio, tubular changes may be a contributing factor in the disease.

Summary. Five cases of renal glycosuria were examined functionally. In 3 of them renal biopsies were obtained. In these 3 cases no structural alterations were found. Alkaline phosphatase activity of the proximal tubules was normal. No alterations in the fine structures of the proximal tubular cells, which would be considered typical of renal glycosuria, could be detected with the electron microscope.



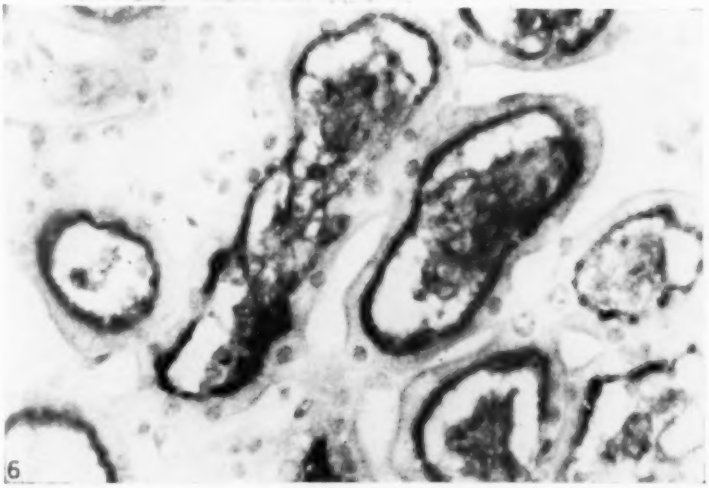


Fig. 4.—Case D.V.V. Renal biopsy. Phosphatase stain. Alkaline phosphatase in proximal tubules.

Fig. 5.—Case S.O. Renal biopsy. Phosphatase stain. Alkaline phosphatase in proximal tubes.

Fig. 6.—Case S.O. Detail of Fig. 4. Alkaline phosphatase in brush-border.

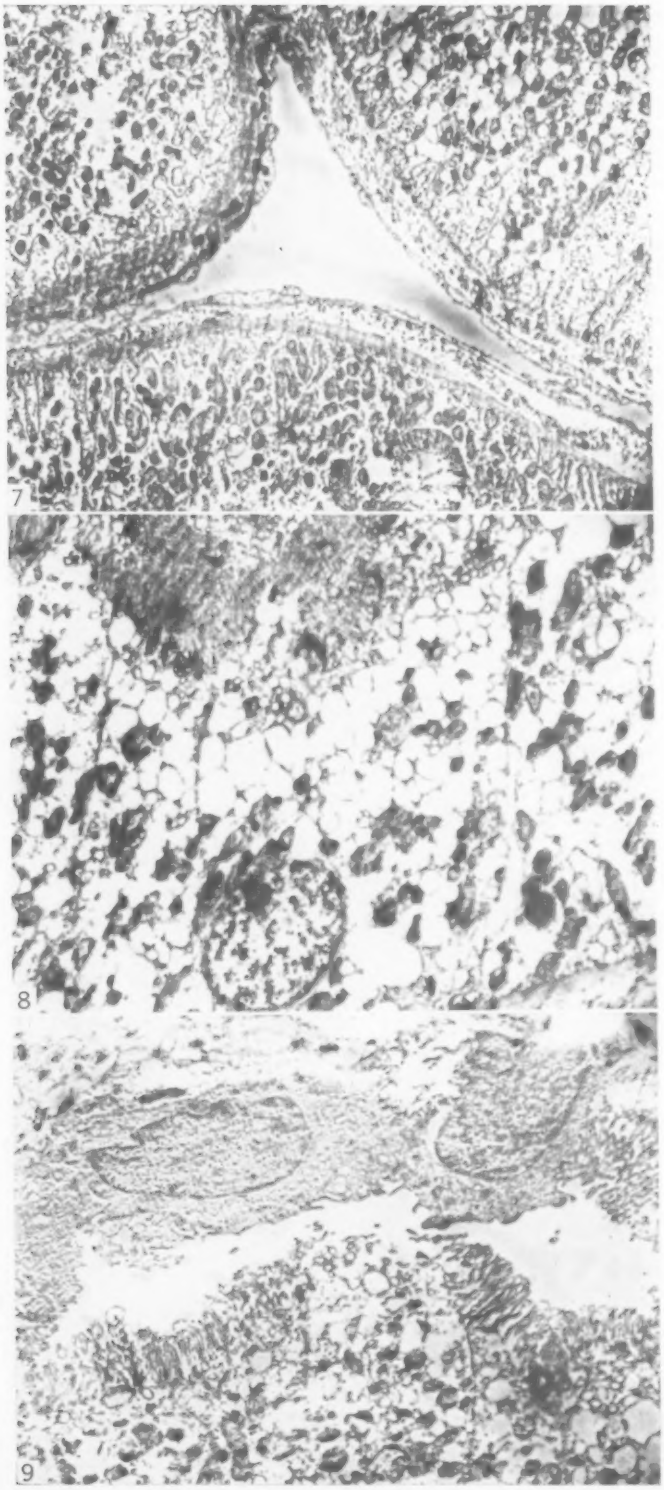


Fig. 7.—Case S.O. Electron micrograph. Part of 3 proximal convoluted tubules.
Fig. 8.—Case S.E. Electron micrograph. Part of proximal convoluted tubular cell. Tightly packed microvilli of brush-border and numerous large vacuoles.
Fig. 9.—Case S.E. Electron micrograph. Lumen of proximal convoluted tubule. Loosely organized microvilli.

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SUMMARIO IN INTERLINGUA

Aspectos Morphologic de Glycosuria Renal

Cinque casos de glycosuria renal esseva examinate functionalmente. In 3, biopsias renal esseva obtenite. In illos, nulle alterationes structural esseva constatate. Le activitate de phosphatase alcalin del tubulos proximal esseva normal. Nulle alterationes del structura fin del cellulas ab tubulos proximal—de character considerate como typic de glycosuria renal—esseva detegibile per medio del microscopio electronic.

THE USE OF THAM* TO PREVENT HYPERVENTILATION AND ACIDOSIS WHILE BREATHING CARBON DIOXIDE

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THAM* is a weak, organic base which has been used for controlling respiratory acidosis, both in dogs (Nahas[5]) and man (Brinkman *et al.*[2]). It combines with carbonic acid in the blood to form bicarbonate, producing a rise in arterial pH. No serious toxicity has so far been described, although in large doses hypoglycemia (Tarail and Bennett[8]) or tetany (Brinkman *et al.*[2]) may occur.

It has already been demonstrated that THAM will temporarily correct the chronic respiratory acidosis which may occur in diffuse obstructive emphysema (Brinkman *et al.*[2]). In such patients, although the carbon dioxide tension ($PaCO_2$) remained relatively unchanged, minute ventilation was depressed as the arterial pH rose to normal or above normal levels, indicating that the respiratory center was apparently more sensitive to a rise in arterial pH than to a fall in the $PaCO_2$. However, in patients with chronic respiratory acidosis, the respiratory center is less sensitive than normal to changes in the $PaCO_2$ (Gray[3]), which may be the reason that in those patients, ventilation responded more readily to change in the arterial pH. It was, therefore, decided to study the effect of THAM in individuals with artificially produced respiratory acidosis in whom there was a normally sensitive respiratory center.

Method: Three young male adults who were convalescent from minimal pulmonary tuberculosis were studied.

*Trishydroxymethylaminomethane.

They were placed in a semi-reclining position, and their expired gas collected in a 300-L. Tissot spirometer, from which was calculated the minute ventilation. A resting arterial blood was drawn (Blood I), and they were then given a mixture of 5% carbon dioxide and 95% oxygen to breathe for 7 to 9 minutes at which time an arterial blood specimen (Blood II) was withdrawn, and the CO_2 inhalation discontinued. Thirty to 60 minutes later, the resting minute ventilation was again recorded, following which inhalation of the 5% carbon dioxide mixture was re-started. At the same time an infusion of 20 to 36 gm. of THAM in 200 ml. of water was given intravenously, this being a dose of 48 to 56 mg. of THAM per kg. per minute. The THAM was administered over an 8- to 11-minute period, at the end of which time another arterial blood specimen was taken (Blood III). The carbon dioxide inhalation was continued for a further 3 to 7 minutes, when a final arterial blood was obtained (Blood IV).

The pH of the arterial blood was measured with a glass electrode and a Cambridge pH meter at 37.4° C. The carbon dioxide content was calculated by the method of Van Slyke and Peters[7]. The carbon dioxide tension was calculated from the Henderson-Hesselbach equation.

Results. In all 3 individuals there was a marked increase in minute ventilation following the inhalation of 5% carbon dioxide in 95% oxygen (see Figs. 1 to 3). However, when THAM was given simultaneously, the ventilation was maintained either at near normal level or depressed. After stopping the THAM infusion, but with continuation of the carbon dioxide inhalation, there was an immediate increase in ventilation, indicating that the effect of THAM is not sustained.

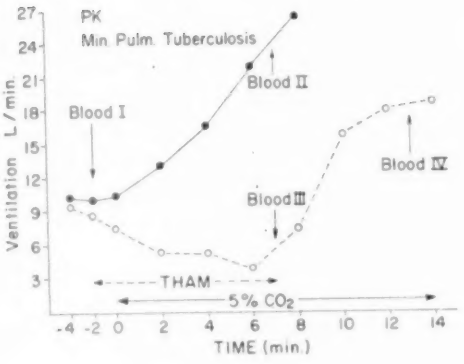

Fig. 1

P.K.	*I.*	*II.*	*III.*	*IV.*
			CO_2 & THAM	*THAM Stopped*
	REST	*5% CO_2 7 min.*	*7 min.*	*6 min.*
pH	7.40	7.35	7.42	7.42
P_{CO_2}	40	45	50	48
C_{CO_2}	21.20	21.50	27.30	26.70
S_{O_2}	96.2	100+	100+	100+
V	10.15	23.4	5.3	17.7

L./min.

P_{CO_2} = carbon dioxide tension in mm. Hg.
C_{CO_2} = carbon dioxide content in mEq.
S_{O_2} = arterial oxygen saturation %
V = minute ventilation in liters per minute.

TABLE 1

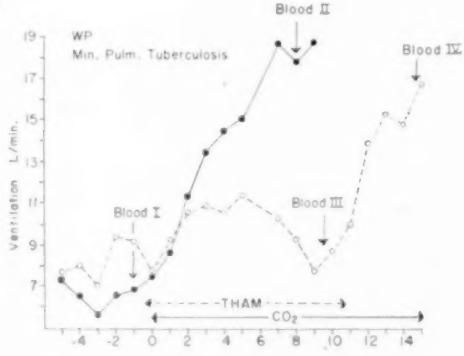

Fig. 2

W.P.	*I.*	*II.*	*III.*	*IV.*
			CO_2 & THAM	*THAM Stopped*
	REST	*5% CO_2 8 min.*	*9 min.*	*3 min.*
pH	7.38	7.38	7.51	7.42
P_{CO_2}	45	45	42.5	50
C_{CO_2}	22.70	22.70	27.60	27.25
S_{O_2}	95.5	100+	100+	100+
V	6.0	18.3	8.5	15.4

L./min.

TABLE 2

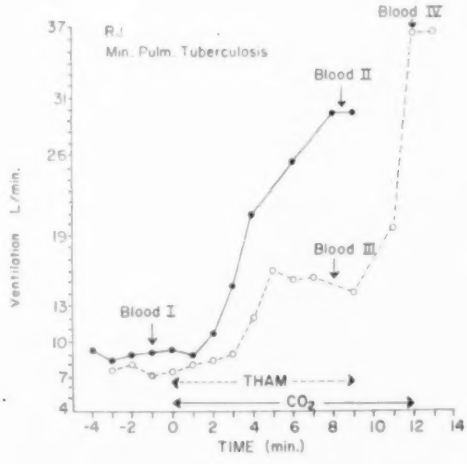

Fig. 3

R.J.	*I.*	*II.*	*III.*	*IV.*
			CO_2 & THAM	*THAM Stopped*
	REST	*5% CO_2 9 min.*	*8 min.*	*6 min.*
pH	7.38	7.28	7.36	7.30
P_{CO_2}	45	57.5	55.5	65
C_{CO_2}	22.50	23.45	26.70	27.45
S_{O_2}	96.5	100+	100+	100+
V	8.4	29.5	14.7	36.5

L./min.

TABLE 3

Figs. 1–3.—Illustrating the changes in minute ventilation as a result of carbon dioxide inhalation and administration of THAM.

The resting arterial blood studies were normal. Following inhalation of the carbon dioxide mixture, the $PaCO_2$ rose as the pH became acidotic in 2 subjects, but in the third (W.P.) there was no measurable change in the arterial blood despite a threefold increase in ventilation. With the simultaneous infusion of THAM during carbon dioxide breathing, the $PaCO_2$ again rose, but the pH became alkalotic due to a concomitant rise in the arterial blood bicarbonate.

Following cessation of the THAM infusion, but with continued inhalation of 5% carbon dioxide, the $PaCO_2$ increased but the pH fell, with the exception of P.K. in whom the arterial blood showed no significant change despite a marked increase in ventilation. The bicarbonate level remained high, being dependent upon urinary excretion for a return to normal levels (Nahas[6]). Coincidental with these changes in the arterial blood, the minute ventilation was markedly increased.

Discussion. These 3 men had a normal response to inhalation of carbon dioxide, with a two- to fourfold increase in minute ventilation. This effect was partially or completely neutralized when THAM was given simultaneously.

The carbon dioxide tension in the alveolar and arterial blood is almost identical, provided there is no abnormal venous arterial shunt. This being the case, when these 3 subjects inhaled 5% carbon dioxide while simultaneously receiving THAM, the $PaCO_2$ and hence the $PACO_2$ (alveolar carbon dioxide tension) increased 2.0 to 10.5 mm. Haldane and Priestley[4] and Alexander and co-workers[1] have shown that it only requires an increase of 1.5 to 1.7 mm. in $PACO_2$ above resting level to double the alveolar ventilation. The alveolar ventilation was not measured in this study, but an increase in the $PACO_2$ of 2.0 to 10.5 mm. would certainly be expected to produce a marked increase in minute ventilation. But such was not the case. The minute ventilation increased only 14 to 18% in 2 subjects while in the third (P.K.), the minute ventilation was reduced 50%, despite a 10 mm. rise in the $PACO_2$. These findings indicate that the expected response to inhalation of carbon dioxide with an increase in $PACO_2$ was modified by the simultaneous administration of THAM.

Gray[3] has shown in metabolic acidosis that a lowering of arterial pH is a weak respiratory stimulant when compared with the increase in ventilation which occurs when breathing carbon dioxide. Gray also demonstrated in metabolic alkalosis that ventilation is only slightly reduced below normal. It would, therefore, appear that change in pH has little effect on the respiratory center, whereas inhalation of carbon dioxide produces a much more profound effect. In the artificial conditions produced in this study, despite the inhalation of 5% carbon dioxide, the pH remained alkalotic and the anticipated increase in ventilation was not observed. This is probably a protective mechanism, as hyperventilation, by eliminating carbon dioxide, would result in further elevation of pH. Instead, as pH rises, ventilation is depressed and this may actually be sufficient to cause a marked fall in the arterial oxygen tension (Brinkman *et al.*[2]). This was not observed in the present study as the subjects were breathing 95% oxygen.

Another possibility is that THAM depresses ventilation due to a toxic effect on the respiratory center. If this were the case, then the effect is extremely short-lived, as shown by the immediate increase in ventilation which follows cessation of the THAM infusion. It is unlikely that the respiratory

center would recover from a toxic state so rapidly.

This study demonstrates a change in the respiratory center in alkalosis whereby it responds primarily to an increase in arterial pH instead of to an elevation in $PaCO_2$. The end result is to prevent further alkalosis developing from hyperventilation.

Summary. THAM can prevent the hyperventilation and acidosis which normally occurs while breathing 5% carbon dioxide. Its mode of action is discussed and it is postulated that in alkalosis, the respiratory center is more responsive to arterial pH than to arterial carbon dioxide tension.

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SUMMARIO IN INTERLINGUA

Le Uso de Trishydroxymethylaminomethano in Prevenir Hyperventilation e Acidosis Durante le Respiration de Bioxydo de Carbon

Trishydroxymethylaminomethano (THAM) pote prevenir hyperventilation e acidosis que occurre normalmente durante le respiration de 5% de bioxydo de carbon. Le modo de action del droga es discutite. Es postulate que in alkalosis le centro respiratori responde plus sensibilemente al pH arterial que al tension arterial de bioxydo de carbon.

TREATMENT OF OCCLUSIVE VASCULAR DISEASE WITH DISODIUM ETHYLENE DIAMINE TETRAACETIC ACID (EDTA)

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AND

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THE frequently disabling but most usually fatal consequences resulting from atherosclerotic occlusion of coronary, cerebral and lower extremity arteries constitute man's major cause of death. This arterial disease process is almost always far advanced when brought to the attention of a doctor. The belief that atherosclerosis was part of the biological aging process has given way to the unproved but more optimistic opinion that it is largely an acquired metabolic disease and subject to reversibility.

Among the several factors that take part in atherosclerosis, certain fats and especially cholesterol have been given major consideration. However, we do not have an adequately controlled study in man to indicate that diet alone effects the subsequent development of atherosclerosis. We are not able to prevent or make an early diagnosis of oncoming complications of this disease but must wait until arteries have become critically occluded. There is no objective or laboratory method for accurately gauging the effect of a drug on atherosclerotic processes so we are forced to appraise therapeutic action by control of symptoms with patients especially under increasing physical stress. The real value of any drug depends on what it does in the hands of a practicing physician and not what it should or should not do on the basis of laboratory experiments.

The atherosclerotic process includes lipids, proteins, polysaccharides, and minerals of which latter the most important therapeutically may be calcium although magnesium and other trace elements may be more intimately involved. The mineral component appeared to be the most favorable avenue for attack on this disease. Many of the human body's minerals give a predictable response to particular, safely usable, chelating agents and therefore the atherosclerotic process could be beneficially altered by binding the body's available ionic calcium and other minerals, and so requiring replenishment from metastatic deposits of calcium and possibly restoring normal competitive balances as with magnesium and re-establishment of necessary mineral enzyme functions. It was our purpose therefore to utilize the principle of chelation on the bound or living calcium and other minerals of the human body in the hope of alleviating the manifestations of angina pectoris, intermittent claudication and cerebral arteriosclerosis.

This report on angina pectoris covers mostly those patients who had had the classical disease syndrome not longer than 3 years but with increasing severity of pain and limitation of physi-

cal activity and others who had a history and electrocardiographic evidence of myocardial infarction followed later by angina pectoris of increasing severity. Bias has been avoided as much as possible in interpreting results. The double blind test for appraising new drugs has been found to be far from infallible and the same can be said for the Master's test. Therefore, our results are based on careful analyses of the amount and type of exertion that was required to precipitate anginal pain before and after therapy, change in electrocardiographic pattern, length of time symptomatic improvement has been retained and percentage of favorable results that have greatly exceeded the known maximum of human response to placebo therapy.

The patients with intermittent claudication had greatly decreased oscillometric readings in their legs, greatly limited walking ability because of induced leg pain, and more than 80% of them had rest pain. They all had the usual signs of general arteriosclerosis and local signs in their legs of lowered skin temperatures confirmed in many by thermo-couple readings and also nutritional changes in skin and toenails.

The patients with cerebrovascular disease fell into 3 categories: (a) acute or old cerebrovascular accidents, (b) severe vertigo or tinnitus and (c) senility, all of them having general and especially ocular signs of advanced arteriosclerosis. The diagnosis of cerebral vascular pathology was supported in many by changes in electroencephalograms.

Method. With greater experience gained since our previous report (Clarke *et al.*[15]) the amount of disodium ethylene diamine tetraacetic acid has been decreased from 5 to 3 gm. in each 500 ml. of 5% glucose or normal saline that has been given intravenously in 2 to 3 hours. This amount was administered on each of 5 consecutive days, omitted 2 days and a rest period of 1 week taken between the first and second series of 15 infusions each. This reduced but equally as effective amount of the compound has largely eliminated local and general side reactions although we have continued to administer tablets of pyridoxine, 25 mg., 3 times daily, during the period of administering infusions. We have continued to administer short booster series of 5 daily infusions of EDTA but greater experience has indicated that the continuing action of EDTA permits the time interval in many patients to be extended from every 6 to 9 or even 12 months. The patients who have had an acute cerebral accident should be at least able to sit up and take nourishment before administering EDTA. This is desirable to avoid possible renal irritation and even elevated blood nonprotein nitrogen which changes, however, are reversible on stopping EDTA.

Results. ANGINA PECTORIS. The differences in diagnostic standards and great individual variability in methods of reporting mortality and therapeutic statistics on angina pectoris minimize the value of comparing results. However, certain facts do become valuable. The natural history of angina pectoris under usual medical management has had an average duration of 4.9 years from onset to death and a 2-year mortality rate of 23.6% (Black *et al.*[9], Sigler[38], Parker *et al.*[32], Thompson and Plachta[45], White and Bland[48] and Tandowsky[44]). The degree of clinical improvement has been negligible (Hugan[23]). There have been different surgical approaches as poudrage using various abrasive materials (Thompson and Plachta[45], Brofman[12]), and this modified (Beck and Leighninger[6]), ligation of the internal mammary artery (Glover *et al.*[20]), implantation of an artery into the myocardium (Vineberg and Walker[47]) and coronary endarterectomy (Bailey[3]) that is too recent for appraisal. The surgical approach has not lengthened life expectancy or given much above the maximum response obtained with placebo therapy. A sum-

mary and comparison of previously reported medical and surgical experiences and our results with 76 patients who had angina pectoris and were treated with EDTA are presented in Table 1. The reported 2-year mortality of 23.6% under past medical management was significantly greater than our

TABLE 1.—BY COMPARING EDTA TREATMENT OF ANGINA PECTORIS WITH THE USUAL EXPECTANT MEDICAL AND VARIOUS SURGICAL METHODS THE GREATEST SYMPTOMATIC IMPROVEMENT AND LOWEST MORTALITY OCCURRED WITH EDTA THERAPY

Patients: 76
Ave. age: 61 years.

Treated with EDTA

Ave. Duration of Disease
Pre-treatment 44 mos.
Post-treatment 28 to 70 mos.

Myocardial Infarction
Ancient 28
Recent 5

Blood Pressure
Normal 57
Elevated 19

Results of Therapy
90 to 100% symptom relief 87%
75% symptom relief 9.2%
No change 3.8%

Recurrence of pain in ave. of 2 years in 20%

Mortality (Myocardial Infarction)
Therapy Completed 4%
Therapy not completed 9%

COMPARATIVE RESULTS

	Onset to Death	*Mortality*	*Clinical Result*			*Recurrence*
			Much Improved	*Improved*	*No Change*	
Medical	4.9 yrs.	23.6% (2 yr.) 53.0% (3 yr.)	0	13%	87%	
Surgical	5 yrs.	—	44%	41%	15%	10 to 25% within 2 years.
EDTA	—	13.0% (2 yr.)	87%	9.2%	3.8%	20% in average of 2 years.

13% under EDTA therapy. It seemed remarkable that our result of 87% of symptomatically improved patients was the same as has been reported for unimproved under expectant medical management. The patients whom we have treated during the past 3 years have had a routine follow-up of 5 daily infusions of EDTA every 6 or 9 months and the recurrence of anginal symptoms has been reduced to less than 20% during 2 years post therapy and this has usually responded to longer booster series of 10 to 15 daily infusions.

INTERMITTENT CLAUDICATION. Our purpose with these patients was to relieve leg pain at rest, prolong walking distances without pain and prevent gangrene. We have treated some patients who already had extensive gangrene of their feet and obviously would require amputation. The EDTA therapy did greatly reduce their leg pain, the run off at time of amputation and healing of the stump were superior to that found in comparable patients operated without previous EDTA therapy.

Among the 31 patients reported 22 had rest pain and many had not slept a full night in bed for weeks; a few had slept in a chair. Relief from rest pain

occurred and was retained in all such afflicted patients. A further therapeutic result observed in all patients was improved ability to walk much longer distances without inducing leg pain. All of these patients had sought help because the leg pain induced by walking had limited their activity so as to threaten their employment. We had no 3-year from onset mortality where Spaulding[41] reports 13%, Allen, Barker and Hines[1], 33% and our 5-year from onset mortality was 10% where Spaulding[41], reports 25%. We had no mortality from the leg vascular disease but like other authors did have from coronary artery disease. The presence of multiple occlusive vascular syndromes in the same patient increases mortality. A comparison of our results in 31 patients after EDTA therapy with statistical reports by others are presented in Table 2.

CEREBROVASCULAR DISEASE. The ages of this group of patients averaged several years older than in our patients with angina pectoris or intermittent claudication. These patients had the last form of occlusive vascular disease to whom we have administered EDTA therapy because of the opinion that brain damage that has been produced by occlusive vascular disease was not subject to corrective measures. The demonstration that true arterial thrombosis may not occur in arterioscle-

TABLE 2.—OF 31 PATIENTS WITH INTERMITTENT CLAUDICATION, 22 OR ALL THOSE AFFLICTED WERE RELIEVED OF REST PAIN BY EDTA THERAPY AND THE 3- AND 5-YEAR FROM ONSET MORTALITY WAS COMPARATIVELY REDUCED

(Treated with EDTA)
Patients: 31

Ave. Duration of Disease		*Results of Therapy*	
Pre-treatment	34 mos.	90 to 100% relieved	23
Post-treatment	27 mos.	75% relieved	4
		No change	1
Status of Disease		Worse	2
Progressive	23	Amputations	2
Rapidly progressive	8	Recurrence of pain after ave. of 22 mos.	4
Rest pain	22	Improved with treatment	3
Existing gangrene	1		
Existing arterial graft	3		
Blood Pressure		*Mortality*	
Normal	23	C.V.A.	1
Hypertensive	8	Myocardial infarction	2

COMPARATIVE RESULTS

Author	*No. of Patients*	*Complications*		*Mortality*	
		Gangrene	*Amputations*	*3 Year*	*5 Year*
Clarke *et al.* (1959)	31	3%	3%	—	10%
Spaulding (1956)	108	—	25% within 5 years	13%	25%
Allen, Barker and Hines (1955)	Not stated	—	"Frequent"	33%	—
Hines and Barker (1940)	116	50%	—	55% (after first visit)	—

rotic vascular disease of the brain (Bruetsch[13]) prompted us to use EDTA therapy in the 25 patients whose records are presented in Table 3. Although this group included 6 patients with acute paralysis and 8 with senility the 3-year mortality has been only 25% and that due chiefly to heart failure. The relief obtained from severe vertigo associated with cerebellar vascular disease with EDTA therapy was outstanding along with unexpected and striking improvement in important mental faculties by those in a state of senility. We have observed patients change from a state of helpless mental confusion to one of satisfactory self sufficiency with renewed control of important physical functions. The paralysis caused by recent cerebrovascular accidents seemed to improve faster and more completely than usually expected and tinnitus, even of long standing, has been relieved. The people with advanced cerebrovascular disease are well along on the path of major disability or death and the subjective and objective signs of improvement with EDTA therapy, in degree and kind, were significant.

TABLE 3.—AMONG A GROUP OF 25 PATIENTS WITH CEREBROVASCULAR DISEASE TREATED WITH EDTA, ALL WHO HAD CEREBELLAR VERTIGO, OR DIZZINESS HAD COMPLETE RELIEF AND THE MANIFESTATIONS OF SENESCENCE WERE SIGNIFICANTLY ALLEVIATED

(Treated with EDTA)

Patients		*Results of Therapy*	
Number of patients	25	Acute paralysis	Good
Average age	66.9 yrs.	Severe vertigo	Excellent
Average followup	37 months.	Old paralysis	Slight value
		Senility	Good
		Tinnitus	Good
Clinical Status		*Mortality*	
Acute paralysis	6	C.V.A.	2
Severe vertigo	7	Congestive heart failure	4
Old paralysis	3		
Senility	8		
Tinnitus	5		

EFFECT OF EDTA ON BLOOD CHOLESTEROL. A significant lowering of blood cholesterol levels in hypertensive patients by intravenously administered EDTA therapy has been reported (Perry and Schroeder[35]). Figure 1 demonstrates the change in daily serum cholesterol levels that occurred in a woman who had angina pectoris and was treated by continuous infusion of EDTA through an indwelling arterial catheter. She received 10 gm. of EDTA during each 24 hours for 6 days. At the end of the first 24 hours of therapy the serum cholesterol level rose slightly and thereafter fell progressively day by day from 300 mg. to 160 mg. per 100 ml. on the sixth day when the infusion of EDTA was stopped after which there was a progressive daily rise in the serum cholesterol levels.

The prolonged effect of EDTA on blood serum cholesterol levels is demonstrated in Fig. 2*A* and 2*B*. The initial fasting serum cholesterol level in all instances was the average of 3 tests that were made at least 1 week apart. In Fig. 2*A* are examples of our general experience with the long time effect of EDTA on the serum cholesterol levels

of patients with signs and symptoms of occlusive vascular disease and elevated serum cholesterol. In one patient the initial serum cholesterol level of over 500 mg. per 100 ml. was reduced by EDTA therapy and remained at a level of 250 mg. per 100 ml., after 18 months. Another patient had an initial of 400 mg. to a maintained level 2 years later of 160 mg. per 100 ml. and in the other it fell gradually from an initial level of 330 mg. to a maintained level of 225 mg. per 100 ml., 30 months later. The sharp rise which in some patients has been preceded by a fall, in the serum cholesterol level with EDTA

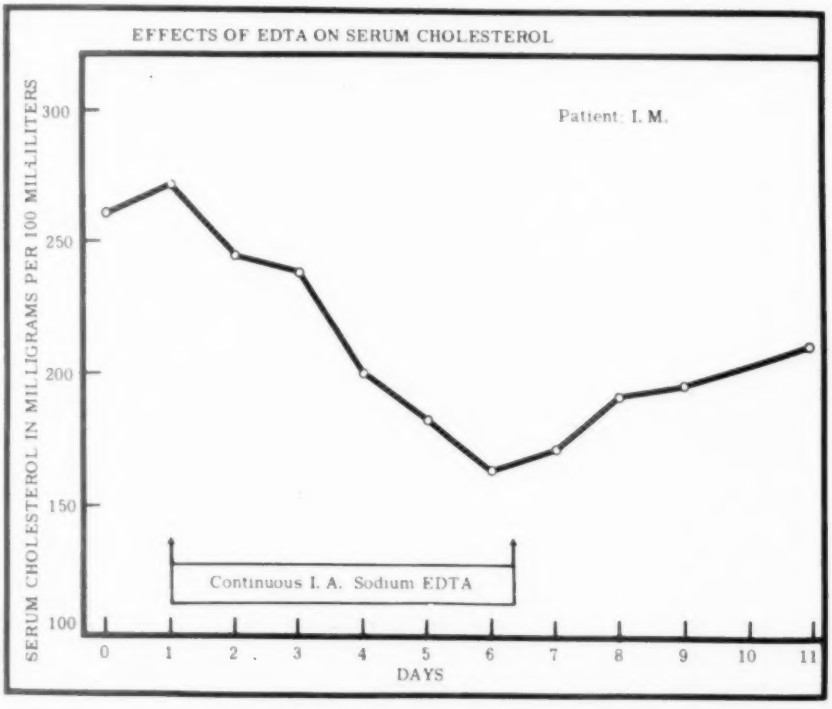

Fig. 1.—With continuous intravenous EDTA therapy serum cholesterol levels were reduced day by day with a total decrease of from 300 mg. to 160 mg. in 6 days. The cholesterol level immediately and progressively rose when EDTA was stopped.

serum cholesterol level of almost 300 mg. per 100 ml. and an unusual rise to 385 mg. per 100 ml. shortly after EDTA therapy was started that was followed by a rapid decline and a maintained average level of 200 mg. per 100 ml., 20 months later. In the 2*B* examples of familial hypercholesterolemia the serum cholesterol of one patient fell gradually from an initial level

therapy suggested the presence of functional derangement or disease of the liver. This type of response is well demonstrated in Fig. 3. This patient had severely crippling occlusive vascular disease in his legs, angina pectoris and cirrhosis of the liver without ascites. His initial serum cholesterol level of about 300 mg. dropped gradually with therapy so that after 15

daily infusions of EDTA it was 210 mg. but then it rose progressively and remained around 360 mg. per 100 ml. After 17 months and 50 gm. more of EDTA the serum cholesterol level dropped and after 6 more months and 25 gm. more of EDTA it declined to the normal level of 210 mg. and was 135 mg. per 100 ml. 2½ years after having started EDTA therapy. Other patients have had this initial fall followed by greater elevation of the serum cholesterol levels with EDTA therapy to lend support to the probability that the effect on serum cholesterol levels in occlusive vascular disease will be influenced in degree and time by the functional condition of

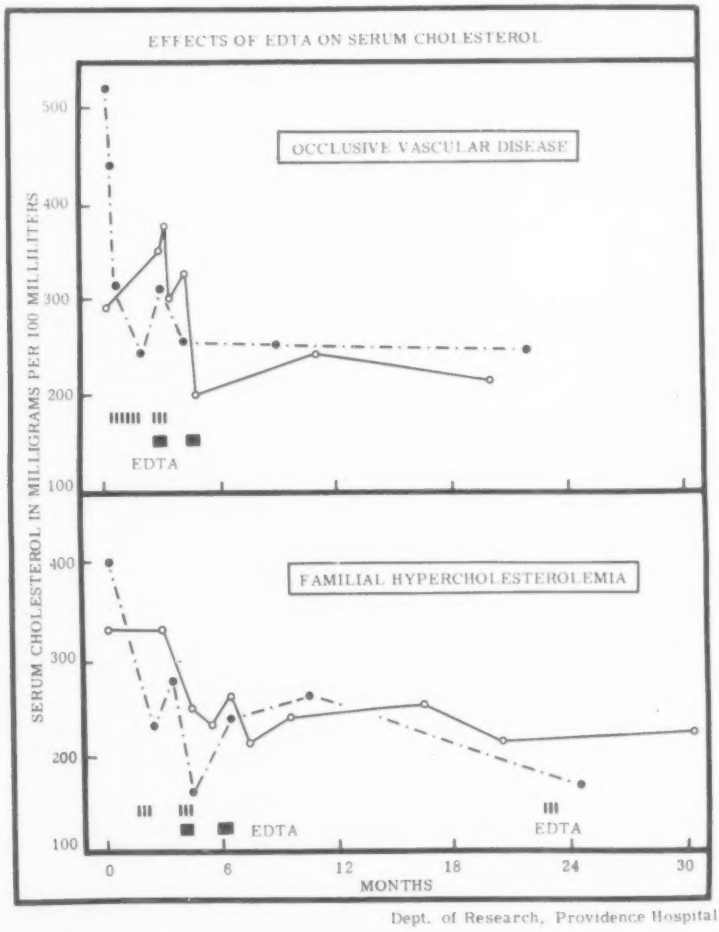

Fig. 2*A*.—In two patients (one solid line, one interrupted line) who had occlusive vascular disease and were treated with EDTA, the elevated serum cholesterol levels were reduced and remained normal for as long as 1.5 to 2 years after therapy.

Fig. 2*B*.—Two patients with familial hypercholesterolemia who were treated with EDTA have maintained normal serum cholesterol levels for from 2 to 2.5 years after therapy.

the liver. The main endogenous source of blood cholesterol is the liver, also of cholesterol catabolism.

Discussion. The prevailing theories and most of the research done in the field of medical therapy for atherosclerosis have been confined to lipid metabolism and its relationship to the formation of atheromatous plaques. When the symptoms of atheromatous occlusive vascular disease appear, the process is advanced and not segmental, as for example, with angina pectoris which seldom occurs unless at least 2 arteries have extensive involvement (McDonald *et al.*[30]).

The lipid or cholesterol part of atherosclerosis is probably, at most, a secondary phase; the cholesterol found in the atheromatous plaque being a product of degenerative reactions already within the arterial wall rather than diffusion from the circulation (Pareira, Handler and Blumenthal[31]). There is no consistent relationship between diet and tissue lipids in humans. In atherosclerosis a thickening of the intima due to thickened fibrocollagenous tissue and localized intimal enlargement at sites of hemodynamic stress antedate alteration in lipids. These local intimal enlargements at points of maximum stress arise from stimulated fibroblasts (Moon and Rhinehart[28]). It is important that atherosclerosis is minimal or absent in

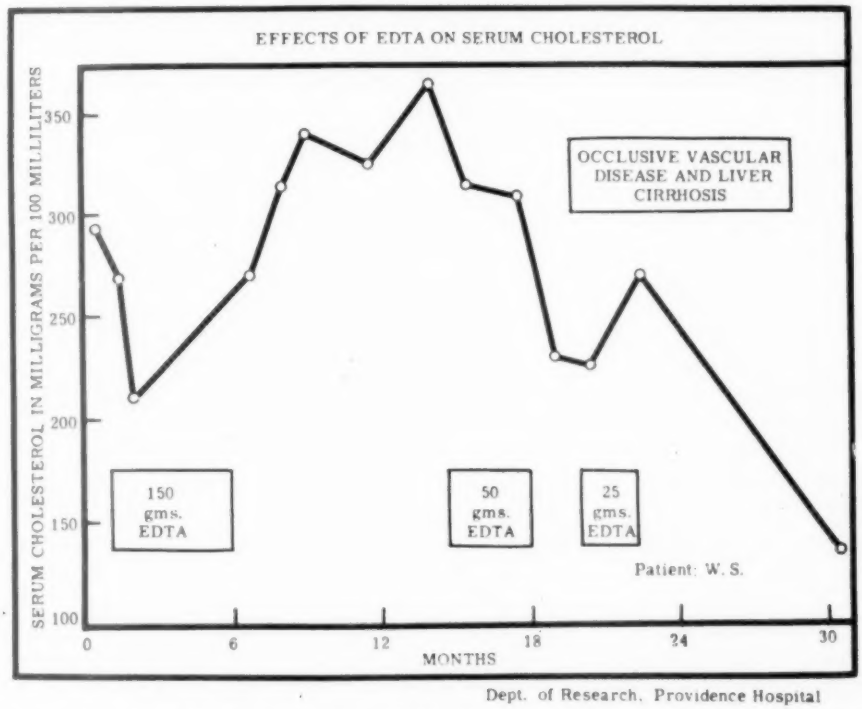

Fig. 3.—The usual fall in serum cholesterol with EDTA therapy was followed by a greater and prolonged elevation with final delayed drop to normal levels in patients with occlusive vascular and chronic liver diseases.

the pulmonary artery unless pulmonary hypertension is present.

The limited role of lipids in atherosclerosis is demonstrated in the not age-connected disease pseudoxanthoma elasticum where the involved skin is characterized by proliferation of fibers in the pars reticularis which stain like elastic tissue and are rich in calcium (Moran and Lansing[29]). A study of over 600 human aortas has demonstrated alterations in the medial elastic tissue and calcium content prior to the appearance of atherosclerosis (Blumenthal, Lansing and Wheeler[11]). The same conclusion was reached with a study of baboons (Gilman and Gilbert[17]). Changes in the medial elastic layer are responsible for subsequent formation of intimal atheromata (Yater *et al.*[30]).

When young arterial walls are injured the integrity of the wall is restored by regeneration. With biologic aging the body can only heal the vessel wall wound with fibrous scar formation, the earliest manifestation being calcification of the vascular elastic membrane and localized accumulation as well as general increase of mucopolysaccharides in the artery wall (Gilman and Hathorn[18]). When heparin was added *in vitro* to the blood of atherosclerotic rabbits the emulsion was broken forming supernatant a thick creamy layer and diminished plasma turbidity. This action was prevented by adding EDTA indicating that calcium ions were needed as well as heparin. It was found that sulfated mucopolysaccharides behave simply to heparin, indicating a relationship between lipid deposition and mineral metabolism especially in connection with polysaccharides of connective tissue (Gilman and Naidoo[19]).

In the biologically aging artery, changes occur in certain colloids which produce splitting and fragmentation of elastic fibers accompanied by calcification of the fragments. Where intimal plaques are present there is intensified duplication of elastic fibrils across the base. These changes are more pronounced in the iliac artery under greatest stress than in the hepatic artery. The degree of calcification is directly proportional to the change in elastic tissue. The elastoid change and calcification in the arterial wall is not uniform but like that of atheromata (Blumenthal, Lansing and Gray[10]). In rabbits the production of medial defects in the aorta influences the deposition of intimal lipids (Anitschkow[2]). The calcium is in, or, on the elastic tissue and consists of an apatite-like crystal. In plants, calcium-like iron may accumulate at the root cell surfaces so that absorption of other elements may be prevented and the plant be destroyed. If the calcium can be kept in a soluble form such as by chelation, the interference and damage can be avoided (Biddulph[8]).

In the healing of a vascular wound that precedes the atheromatous plaque, sulfated mucopolysaccharides play a critical role and chiefly chondroitin sulfate which has a high affinity for calcium (Gilman and Hathorn[18]). The healing vascular area or plaque takes on a Ca:P ratio much like normal bone (Bauer, Marble and Bennet[5]) and similarly in like ratio contains citric acid (Buffa and Colajanni[14]). The participation of the citric acid cycle in the metabolism of arterial tissue seems certain (Laursen and Kirk[26]). With biologic aging the altered vascular elastic fibers serve as a matrix for the deposition of calcium phosphate conditioned by local pH (Lansing[25]).

A disturbance in the relative concentrations of enzymes within arterial tissue may occur in atherosclerosis. The effect of age on the overall metabolic activities and the concentration of

specific enzymes in arterial tissue seems to follow the same pattern of changes that occur in other tissues.

It has been established that the movement of cations such as calcium through cell walls of tissue membranes depends upon the presence of enzyme systems. For example, the transport of sodium through the skin of a frog is blocked by substances which inhibit the enzyme activity. With age comes an imbalance of concentration of enzymes within the arterial wall due to loss of equilibrium between competing essential free ions. The cation forms specific chelates with the protein of specific enzymes (Ussing[46]).

A most important function of chelation in human and animals has to do with conversion of one type of amino acid into another type or transformation of a keto acid into an amino acid, thereby maintaining balances of amino acids in the body when there is the slightest lack of a vital amino acid for a particular purpose such as healing vascular injury. The vitamin pyridoxine here assumes the role of the intermediate and forms chelates with various minerals to accomplish the needed conversion. The enzymes used are transaminases (Williams and Nerlands[49]). The 2 phosphorylated derivatives, pyridoxal phosphate and pyridoxamine phosphate function as coenzymes for transaminase (Meister[27]). An investigation of several transaminases suggested that a pathological link exists between serum enzymatic activity and certain types of obliterating vascular disease (DiSimone and Ferrante[16]). The sulphur content of the aorta of humans increases with arteriosclerosis and the calcium and phosphorus increase as well but in much greater degree in areas of atheromata (Helvelke[22]). It has been demonstrated that a pyridoxin deficiency in the monkey produces atherosclerosis perhaps by specific metal deficiency in the pyridoxin-enzyme system (Schroeder[39]). It has been found that manifestations of scleroderma may be normalized by correcting the abnormal amino acid pattern through chelation of excess calcium or zinc, the derangement of which had blocked the normal functional interrelationship between magnesium and pyridoxal phosphate (Price *et al.*[37]).

It has been suggested that the occlusive vascular diseases may be initiated by the accumulation of trace metals over many years through exposure to gases, treated drinking water, pipes and metal food containers with cumulative poisoning of enzyme systems that are required for regeneration of vascular tissue (Schroeder[40]). The possible excess of calcium could depress the availability of magnesium. The high fat diet has been indicted as causing atherosclerosis and the rarity of heart disease among Bantu tribesmen been quoted as an example. However, the serum magnesium level of the Bantu is significantly higher than in Europeans and a correlation exists between serum magnesium and serum cholesterol levels. In monkeys with pyridoxine deficiency induced atherosclerosis and hypercholesterolemia the administration of pyridoxine increased the serum magnesium level (Bersohn and Oelofse[7]). Some have claimed symptomatic relief and return to normal of biochemical abnormalities in people with angina pectoris who were treated with intramuscular injections of magnesium (Parsons[33]). A high fat diet might help to create a magnesium deficiency. This has been observed in rats with the development of atherosclerotic lesions that could be prevented by greatly increasing magnesium intake (Hellerstein *et al.*[21]). The injection of calcium into magnesium deficient dogs caused calcification of

the heart and larger arteries that magnesium deficiency alone did not produce (Syllm-Rapoport and Strassburger[43]).

Apparently, tissue calcium increases and magnesium decreases with aging. This change in Ca:Mg ratio, with more ionic calcium available may follow the normal aging changes in kidneys that produces declining ability to maintain proper acid base equilibrium, a state that is magnified by the shrinking capacity of the lungs to control carbon dioxide requirements (Pitts[36]). The increasing availability and shift of calcium to soft tissues with biologic aging and atherosclerosis may be part of the increasing prolonged exposure of the distal convoluted kidney tubules to concentrated metabolites with depression of the renal carbonic anhydrase and partial suppression of the acidifying mechanism (Balotin[4]).

For whatever part an elevated serum cholesterol may play in atherosclerosis the chelating agent EDTA can significantly reduce serum levels in humans (Perry, Mitchell and Schroeder[34]) also in induced atherosclerotic pigeons where it also decreases the intensity of atherosclerotic changes (Supniewski, Chrusciel and Chrusciel[42]). Calcium influences the colloids of cells more than any other cation. The cell cortex of involved arterial tissues when weakened allows more calcium to enter and cause degenerative changes such as are found in atherosclerosis (Lansing[24]).

The role of lipid metabolism and cholesterol has been studied extensively without leading to an acceptable explanation of atherosclerosis or providing a worthwhile therapeutic approach to the disease. We err when atherosclerosis is thought of as an acquired metabolic disease greatly related to a high fat diet and subject to much reversibility. In our opinion dietary fat or fat metabolism plays but a minor or contributing role in atherosclerosis. The complications of atherosclerosis can be beneficially influenced by changing mineral-enzyme systems with the chelating agent EDTA. The EDTA offers not only a mode of therapy for an important disease but represents a new approach toward the therapy of disease in general through control of basic and fundamental chemical processes. These are the enzymes that control all biochemical processes and are sensitive to concentration of specific metals and can be controlled through providing or withholding the metal required. The effective ionic concentration of many metals may be controlled through the presence of complexing agents and EDTA is one of an unlimited series which can operate by means of chelation and the multiplicity of possible biological effects is seemingly endless.

Summary. The lack of diagnostic measures for atherosclerosis, also of objective laboratory controls for interpreting therapy have been emphasized. As the occlusive vascular syndromes are largely symptomatic diagnoses, so must therapy be judged by its influence on such symptoms.

While lipids, proteins, and polysaccharides are part of atherosclerosis, the most logical therapeutic approach appears to be through the essential minerals. For this purpose the chelating agent ethylene diamine tetraacetic acid (EDTA) was administered intravenously with the chief immediate action on bound or living calcium.

The results of such therapy and comparisons with other methods are presented for 76 patients with angina pectoris, 31 patients with intermittent claudication, and 25 patients with various manifestations of cerebrovascular disease.

The ability of EDTA to produce a sustained lowering of abnormally high

serum cholesterol levels in patients with familial and occlusive vascular disease hypercholesterolemia has been demonstrated.

The various theories of atherosclerosis have been briefly considered with arguments presented against the importance of fats and cholesterol and in support of these changes in arteries as part of the biological aging process and particularly on the importance of the arterial elastic layer, calcification, magnesium, and altered mineral, enzyme systems with the suggestion that they might be initiated by change in body pH brought about by aging changes in the kidneys.

The positive action of EDTA in this series of events with possible restoration of normal Ca:Mg balance, withdrawal of calcium, and stabilization of essential mineral enzyme systems have been discussed.

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SUMMARIO IN INTERLINGUA

Le Tractamento de Occlusive Morbo Vascular con Acido Disodio-Ethylenediamine-Tetraacetic

Le manco de tests diagnostic de atherosclerose e de objective methodos laboratorial in le evalutation therapeutic es sublineate. Como le diagnose del syndromes de occlusion vascular depende in grande mesura del symptomas, assi etiam le therapia debe esser judicate per su effecto in le symptomas.

Durante que lipidos, proteinas, e polysaccharidos es parte de atherosclerose, le plus plausibile methodologia therapeutic pare basar se super le minerales essential. Pro iste ration, le agente de chelation, acido ethylenediaminetetraacetic, esseva administrate per via intravenose con le major effecto immediate super le calcium ligate o vive.

Le resultatos de iste typo de therapia e comparationes con altere methodos es presentate pro 76 patientes con angina de pectore, 31 patientes con claudication intermittente, e 25 patientes con varie manifestationes de morbo cerebrovascular.

Le capacitate del agente de effectuar un perdurante reduction de anormalmente alte nivellos seral de cholesterol in patientes con le hypercholesterolemia de morbo vascular familial e occlusive ha essite demonstrate.

Le varie theorias de atherosclerose es considerate brevemente. Es presentate argumentos contra le importantia de grassias e de cholesterol. Es arguite in favor del these que le alterationes arterial in question es parte del processo de de invetulation biologic. Factores sublineate es le strato elastic del arterias, le

(Continued on page 681)

PLASMA 17-HYDROXYCORTICOSTEROID LEVELS IN NORMOTENSIVE AND HYPERTENSIVE SUBJECTS AND THE INFLUENCE OF POSTURE*

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Many studies have been reported concerning the role of the adrenal cortex in various types of hypertension, both animal and human (Wakerlin[12]). Some reports indicate that areas of adrenocortical hypertrophy and hyperplasia appear more frequently in hypertensives than in normals. In many hypertensive patients, complete adrenalectomy with restricted hormone therapy has produced a significant blood pressure reduction. Deoxycorticosterone acetate has been shown to produce a greater chronic pressor effect in essential hypertensives than in normotensives (Perera[7]).

Urinary output of formaldehydogenic steroids has been reported both as increased (Corcoran, Page and Dustan[2]), and as unchanged (Tobian[11]), in essential hypertensives as compared with normotensives. Urinary glucocorticoids measured by biologic assay have also been reported as increased (Selye[9]) or unchanged (Hetzel and Hine[4]). The finding (Genest *et al.*[3]) that hypertensives excrete larger amounts of aldosterone than normals suggests a possible relationship between hypertension and adrenocortical dysfunction. Recent work (Cooper *et al.*[1]) with incubated adrenal tissue from hypertensive subjects points to an abnormal pattern of corticosteroid formation.

Previous studies of glucocorticoid levels in essential hypertension have been largely confined to urinary measurements and have yielded discordant findings. In view of the absence of data, except in an occasional subject, on blood glucocorticoids in essential hypertension the present study was undertaken to compare plasma 17-hydroxycorticosteroid (17 OH CS) levels of normotensive subjects with those of chronic, benign, treated and untreated essential hypertensives under the following conditions: a) during a related

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study of renal function before and after the stress of exercise or the cold pressor test, b) under more normal conditions of ambulation, that is, without renal clearances or other stress; in this latter circumstance, the role of normal diurnal variation and the effect of a postural change to the supine position were also studied.

Methods. GROUP I. These patients undergoing renal function studies consisted of 12 normotensives and 23 essential hypertensives from Temple University Hospital. Of the hypertensives, 10 were receiving reserpine and 13 were without medication at time of study. All patients were without breakfast and were hydrated by drinking 500 ml. of water. Bladder catheterization was performed and 15 minutes later, prior to injection of Inulin or PAH, a blood sample was drawn into a syringe moistened with heparin. The cold pressor test (immersion of one hand in water at 4° C. for one minute) was imposed 1 hour later and ½ hour following the cold application (at the end of the renal clearance study) a second blood sample was obtained. The blood was centrifuged and plasma 17-hydroxycorticosteroids determined by the method of Silber-Porter[10].

GROUP II. Steroid measurements were also made in a smaller series of patients (4 hypertensives and 3 normotensives) undergoing a similar renal study except that exercise in the form of situps was employed instead of the cold pressor test.

GROUP III. In this group consisting of 10 normotensive volunteers and 12 treated essential hypertensives, a comparison of steroid levels was made under normal conditions of ambulation. None was fasted or subjected to renal clearance, exercise or cold stress and all remained ambulatory. Of the hypertensives, half were receiving phenobarbital and half, reserpine. Since the steroid levels were almost identical despite the difference in medication, the values for the entire hypertensive group have been combined.

Because of the similarity in initial steroid levels and in the pattern of response in both normotensives and hypertensives of the previous groups, the role of normal diurnal variation (Migeon *et al.*[5]) was tested in normotensives only. In this group a second blood sample was drawn 1½ hours after the first, the subjects having remained ambulatory during this standard time interval.

GROUP IV. In this group of 10 normotensives and 10 hypertensives the initial conditions were the same as described for Group III except that the hypertensives were without medication. A comparison between normotensives and untreated hypertensives was thus afforded under normal conditions of ambulation. All subjects were then requested to lie supine (as had all renal clearance subjects) for 1½ hours and at the end of this period the second blood sample was taken.

All groups were generally comparable as to age, color and sex. All initial blood samples were drawn between 9:00 and 10:00 a.m.

Results. GROUP I. The initial plasma levels of 17 OH CS in the three categories of this renal clearance test group were similar (Table 1) with no statistically significant differences among them. They are somewhat higher than the initial levels obtained in Groups III and IV in which no renal clearance was being performed.

Steroid values 1½ hours later revealed changes in the direction of decreasing levels (Table 1). These changes were significant and comparable for the three sub-groups.

GROUP II. The steroid levels and changes are tabulated in Table 1 and although the series is too small to afford a valid statistical comparison, the data indicate a close similarity to the results obtained with Group I.

GROUP III. The initial levels for normotensives, while slightly higher than those reported by some investigators (Wallace, Christy and Jailer[13]), compare favorably with values reported by Persky *et al.*[8]. In both normotensive and treated hypertensive groups the values are again quite similar (Table

2). Steroid levels obtained in the normotensive group after an interval of 1½ hours revealed no appreciable change from initial values as occurred in both normotensives and hypertensives of Groups I and II.

Discussion. Insofar as plasma free 17 OH CS levels are concerned, there are no differences between normotensives and treated (as described) or untreated hypertensives under normal conditions of ambulation or under conditions of impending renal clearance studies. In the latter circumstance the higher initial levels observed in both normotensives and hypertensives may very well reflect apprehension or the fasting state, or both.

TABLE 1.—PLASMA 17 OH CS (μg. PER 100 ml. MEAN ± STANDARD ERROR) IN SUBJECTS UNDERGOING RENAL CLEARANCE BEFORE AND AFTER COLD OR EXERCISE STRESS

Group I	*No. of Subjects*	*Before*	*Stress*	*After*	*Diff.*	*% Change*
N	12	28.2 ± 2.4	Cold	18.4 ± 2.1	− 9.8*	−35
H	13	23.7 ± 1.6	"	14.5 ± 1.7	− 9.2*	−39
H-R	10	25.3 ± 2.6	"	16.8 ± 2.4	− 8.5*	−34
Group II						
N	3	29.6 ± 6.7	Exercise	20.1 ± 4.8	− 9.5†	−32
H	4	25.0 ± 4.5	"	14.4 ± 4.4	−10.6†	−42
Time (hrs.)		0	1	1.5		

N = normotensives
H = hypertensives without medication
H-R = hypertensives receiving reserpine
* = statistically significant change ($p = <0.01$)
† = see text under results

TABLE 2.—THE INFLUENCE OF THE SUPINE POSITION ON PLASMA 17 OH CS (μg. PER 100 ml., Mean ± STANDARD ERROR) IN SUBJECTS NOT UNDERGOING RENAL CLEARANCE OR OTHER STRESS

Group III	*No. of Subjects*				*Diff.*	*% Change*
N	10	20.2 ± 2.3	←ambulatory→	18.8 ± 2.1	−1.4	− 6.9
H-M	12	18.7 ± 1.7				
Group IV						
N	10	18.2 ± 1.3	←supine→	10.4 ± 1.6	−7.8*	−43
H	10	15.1 ± 1.7	←supine→	8.6 ± 1.7	−6.5*	−43
Time (hrs.)		0		1.5		

N = normotensives
H-M = hypertensives receiving phenobarbital or reserpine (see text under methods)
H = hypertensives without medication
* = statistically significant change ($p = <0.01$)

GROUP IV. The close similarity in initial steroid levels is again apparent (Table 2) for normotensives and, in this case, untreated hypertensives. Following the 1½ hour reclining period, a significant and comparable reduction occurred. The percent change is identical in both groups and approximates that observed in the renal clearance Groups I and II.

The decreases in steroid levels occurring in all renal clearance patients despite the added stress of exercise or cold suggest that the stimuli of cold or exercise as employed here may have been ineffective in inducing pituitary-adrenal activation. The possibility that these added stresses might have contributed to an increased destruction or utilization of steroid was tested in a preliminary study of patients undergoing renal clearance but with cold or exercise omitted; the same pattern of decreasing levels was again observed. The factor of diurnal variation (Migeon *et al.*[5]) which is normally manifested by a gradual decline in 17 OH CS from morning levels was discounted since ambulatory normotensives of Group III showed no significant change in steroid levels during 1½ hours of normal activity between collection of the first and second blood samples.

The fact that the changes occurred in the clearance groups in which starting plasma levels were higher than nonclearance groups, could mean that over a period of 1½ hours these high levels had simply returned to the more normal resting values such as were found in Groups III and IV at zero time. However, another variable that might have contributed to the observed decreases was positional change. The patients undergoing the renal clearance procedure had necessarily remained supine during the experimental period whereas the subjects in Group III, showing no appreciable change during this period, had remained ambulatory.

The study in Group IV eliminated the renal clearance procedure and obviated high initial steroid values but incorporated the positional change which was an underlying factor in the clearance studies. The finding that even with normally low starting levels, a significant and similar decrease again occurred in both normotensive and hypertensive groups strongly suggests that the postural change or the accompanying physical inactivity, or both, contributed materially if not entirely to the reduction in steroid levels observed in all of the groups (clearance or not) in which the supine position was assumed during the test period.

Muller, Manning and Riondel[6] have reported that the day and night pattern of urinary excretion of aldosterone in normal subjects can be altered by a change from the usual vertical posture and activity during the day to the inactive sitting or horizontal position. A disturbance in excretion of 17 OH CS occurred on the first day of inactivity, the usual pattern of a greater day than night excretion being abolished. However, on subsequent days with continued inactivity, the normal pattern was again observed. This temporary change in 17 OH CS excretion was in contrast to the more sustained alterations in aldosterone excretion under the conditions described. Muller and co-workers concluded that the diurnal pattern of urinary excretion of 17 OH CS is relatively independent of muscular activity and position.

Since significant reductions in plasma 17 OH CS levels can occur upon assuming the supine position for relatively brief periods, the variable of posture in addition to normal diurnal changes should therefore be considered in the interpretation of results obtained following procedures that appear to raise or lower plasma 17 OH CS levels.

Summary. Plasma 17-hydroxycorticosteroid levels in normotensive subjects have been compared with those in treated and untreated essential hypertensives under conditions of renal clearance, before and after the stress of exercise or the cold pressor test. Initial levels were high but similar in all groups. Levels following exercise

or cold declined significantly and in a comparable manner in all groups.

Under more normal conditions of ambulation (without renal clearance or other stress) initial 17 OH CS levels were lower in both groups but again very similar. In this series the possible role of normal diurnal variation during ambulation was tested in the normotensive group but no appreciable change in steroid levels occurred during the standard time interval between collection of blood samples. In both normotensives and hypertensives, however, a significant decline occurred when the subjects were rendered supine for the standard 1½ hour experimental period. Consideration should therefore be given to positional change in the interpretation of data dealing with plasma 17 OH CS in human subjects.

ACKNOWLEDGMENT: The authors acknowledge the able technical assistance of Mr. Jasper P. Reed.

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SUMMARIO IN INTERLINGUA

Le Nivellos de 17-Hydroxycorticosteroide del Plasma in Subjectos Normo- e Hypertensive e le Influentia del Postura

Le nivellos de 17-hydroxycorticosteroide del plasma in subjectos normotensive esseva comparate con illos in patientes con tractate o non tractate hypertension essential, sub conditiones de clearance renal e ante e post le stress de exercitio o le test pressorpi per frigido. Le nivellos initial esseva alte sed simile in omne le gruppos. Post exercitio e post frigido le nivellos descendeva significativemente e de maniera comparabile in omne le gruppos.

Sub conditiones plus normal de ambulation (sin clearance renal o altere formas de stress), le nivellos initial de 17-hydroxycorticosteroide del plasma esseva plus basse in ambe gruppos e de novo multo simile. In iste serie le rolo possibile normal variationes diurne durante ambulation esseva studiate in le gruppo

(Continued on page 689)

ASSOCIATION OF CYANOCOBALAMIN DEFICIENCY WITH MYELOPROLIFERATIVE STATES: REPORT OF THREE CASES

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AND

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Myeloproliferative disorders as conceived by Dameshek[6] are diverse manifestations of the same basic derangement, encompassing erythremia, myelocytic leukemia, myeloid metaplasia and myelofibrosis. This viewpoint has been favorably accepted, particularly as applied to polycythemia vera (erythremia), which ordinarily manifests excessive leukocyte and platelet production along with erythrocytosis. Myeloid metaplasia is considered to exist in nearly all stages of erythremia, and if the patient with this disease survives its thrombotic and hemorrhagic tendencies, myelofibrosis or myelocytic leukemia may eventually supervene. Dameshek's original editorial discusses somewhat analogous observations made with reference to primary myelocytic leukemia.

Certain myeloproliferative conditions, particularly erythremia, allow prolonged survival, and thus the possibility of coexisting disorders which might modify the abnormal bone marrow activity. Among such coincident situations is the chance combination of myeloproliferation with pernicious anemia.

The present paper describes a patient with polycythemia vera who developed a macrocytic anemia, corrected after administration of liver extract. Two additional cases are presented in which cyanocobalamin deficiency appeared to exist in the same individual as did other forms of myeloproliferation. Observations with these patients, and a few similar instances in the literature, bear upon the possibility of cyanocobalamin deficiency influencing the course of myeloproliferative states.

Case Reports. CASE 1. This 64-year-old Jewish man was studied early in 1940 because of recurrent ecchymoses. He was discovered to have marked erythrocytosis, with red blood cells numbering 8,910,000 and the hematocrit 69.2%. The leukocytes numbered 13,600 per c.mm. with 94% neutrophils, none of which were immature forms. Platelets appeared to be increased on the blood film.

Ruddy complexion and hepatosplenomegaly were present. A diagnosis of polycythemia vera was made. Following multiple venesection, normal red cell count and hematocrit levels were obtained.

During the following 2 years, occasional further venesection was done for rising erythrocyte levels (see Fig. 1). In June, 1942, he noted diminution in appetite, energy and sense of well-being. Physical examination at this time showed pallor. Hepatosplenomegaly was at approximately the same degree as 2½ years before. On hematologic study, there was now striking reduction in red blood cells and hematocrit, to 2,060,000 per c.mm. and 26%, respectively. The white blood cell count was 6,700 per c.mm. On smear, there was poikilocytosis and macrocytosis of the erythrocytes.

Gastric analysis showed no free hydrochloric acid after injection of histamine. The mean corpuscular volume of the red cells had risen to 122 cubic micra.

Therapy with liver extract was associated with a significant reticulocyte response, and a rise in red blood cells and hemoglobin (see Fig. 1). The mean corpuscular volume declined from 122 cubic micra to 81 cubic

micra, when the red cell count reached 6,100,000 per c.mm. in October.

In November, 1942, enucleation of a benignly hypertrophied prostate gland was necessitated by complete obstruction of urinary flow. Pyelonephritis developed shortly after operation. This failed to respond to treatment, and the patient died on the seventh postoperative day. Postmortem study included

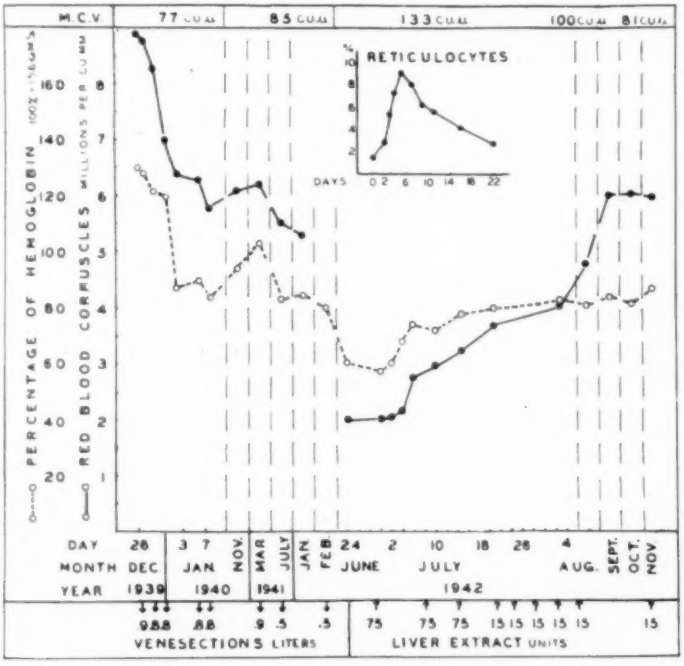

Fig. 1.—Erythrocyte and hemoglobin levels for Case 1.

demonstration of normal red blood cell maturation in the bone marrow, and of myeloid metaplasia in the spleen.

COMMENT. In the foregoing case, polycythemia vera was present for at least 2 and probably 4 or more years, after which the patient became anemic. The erythrocytic response following liver therapy was in every way comparable to that in uncomplicated pernicious anemia. It is therefore felt that this sequence of events indicates the appearance of pernicious anemia during the course of erythremia. All ascertainable features of the latter condition, except hepatosplenomegaly, disappeared until treatment for pernicious anemia was instituted. The patient's death prevented observation as to whether the erythrocytic response with liver would eventuate in erythremic blood levels; this possibility is, however, implied by the red blood cell and hemoglobin indices observed before the terminal illness.

CASE 2. This 83-year-old woman of Anglo-Saxon ancestry was considered to have classical pernicious anemia in relapse, when first studied at the Framingham Union Hospital in September, 1956. Hematologic indices were as shown in the accompanying chart. Physical examination showed pallor, shiny tongue with smooth edges, a grade I systolic murmur at the cardiac apex, and absence of vibratory

sensation below the iliac crests. Knee and ankle jerks could not be obtained, and gait was ataxic. There were no enlarged abdominal viscera.

Bone marrow aspirate was megaloblastic. Peripheral blood smear showed poikilocytosis, macrocytosis, "hypersegmented" polymorphonuclear leukocytes, and decreased platelets. Gastric analysis revealed no free acid after histamine.

Response to therapy was considered typical for pernicious anemia (see Fig. 2).

left but no abnormal forms. The red cells also appeared normal on smear.

This increase of all myeloid elements in the circulating blood was thought to indicate a myeloproliferative state, not clearly classifiable as either leukemia or erythremia. Busulfan (Myleran) was employed in treatment, with a coincident decrease of all formed elements in the peripheral blood (see Fig. 2).

The spleen had not been palpable until the episode considered to be splenic infarction, at which time it was 10 cm. below the

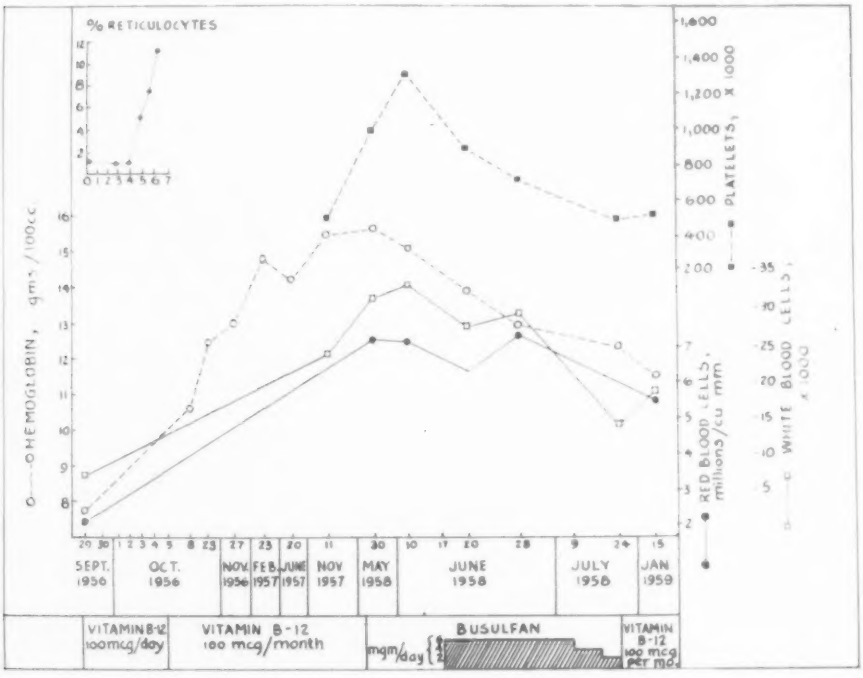

Fig. 2.—Erythrocyte, hemoglobin, white blood cell and platelet levels for Case 2.

During the ensuing 18 months, she did fairly well, though ataxia failed to improve. In this interval there were two or three severe ecchymoses unassociated with trauma. A platelet count in November, 1957, was 500,000. In May, 1958, she re-entered the hospital because of physical findings considered to represent infarction of the spleen. Platelets at this time were increased to 1,100,000 per c. mm., red blood cells numbered 7,000,000 per c.mm. and white blood cells 32,000 per c.mm. with a shift to the costal margin. It remained approximately this size thereafter.

In December, 1958, alkaline phosphatase histochemistry of circulating white blood cells was 100% or 4 plus.

COMMENT. In this elderly woman, reasonably well demonstrated pernicious anemia showed a typical response on treatment with cyanocobalamin. Massive spontaneous ecchymoses

occurred on several occasions after initiation of this therapy, and after 20 months of treatment, apparent splenic infarction led to discovery of a peripheral blood picture in which all myeloid elements were increased, the platelets extraordinarily so.

The usefulness of the concept, "myeloproliferative syndrome" is apparent in this case. The clinical course, with spontaneous ecchymoses and thrombosis, was consistent with erythremia, but in laboratory data thrombocytosis was far more striking than was erythrocytosis. The high alkaline phosphatase of circulating white blood cells, plus the benign clinical course, would seem to argue against myelocytic leukemia at this time.

The response to busulfan was gratifying. It was felt necessary also to continue vitamin B_{12} in fairly large doses, because of combined system disease.

CASE 3. A woman of Irish extraction was first noted to have enlargement of the spleen to below the iliac crest at the age of 60, when she was studied at the New England Center Hospital. The peripheral blood at this time showed no definite abnormality, the hemoglobin being 15 gm.%, the erythrocytes 4,980,000 per c.mm. and the leukocytes 12,300 per c.mm. with a normal differential. Two attempts at sternal aspiration failed to yield marrow.

She received little medical attention thereafter until 9 years later, in March, 1957, when she entered the Framingham Union Hospital complaining of easy fatigability, anorexia, weight loss of 20 lb., and frequent bouts of nausea, vomiting and diarrhea. On physical examination she was slightly pale, but with striking telangiectasia of the face and neck. There was splenomegaly to an enormous degree, coinciding with the description of 9 years before. Deep tendon reflexes were normal, and vibratory sense was not impaired. The liver edge was felt just below the costal margin. Laboratory findings on admission included the hemoglobin of 11 gm.%, an erythrocyte count of 3,750,000 per c.mm. and hematocrit of 34%. Anisocytosis, poikilocytosis and polychromatophilia were noted on the blood smear. The mean cell volume was 92 cubic micra. The leukocyte count was 8,600 per c.mm. No free hydrochloric acid was found in the gastric secretion after histamine.

The patient was discovered to have radiologically evident gallstones, and cholecystectomy was performed on the tenth hospital day. At operation the spleen, though greatly enlarged was not unusual in color or consistency. Biopsy of the spleen showed myeloid metaplasia. The patient refused bone marrow biopsy.

The smooth tongue, achlorhydria and slight anemia led to a trial of vitamin B_{12} with a prompt reticulocytosis as shown in the accompanying chart, and a later rise in hemoglobin, hematocrit and red blood cell count. In October, 1957, this patient's uptake of radioactive cobalt was studied, using a technique described by Schilling[26]. With no evidence for significant renal insufficiency, only 3% of orally administered Co_{60} was recovered in a 24-hour urine collection.

In June, 1958, the patient described her appetite, health and strength as excellent. There had been no diarrhea or vomiting in the 15 months since her hospitalization. The spleen did not appear to have changed in size. Hematologic findings were as noted in the chart (Fig. 3).

COMMENT. This patient is considered as having some form of myeloproliferation, on the basis of enormous splenomegaly which appeared due to myeloid metaplasia. Bone marrow fibrosis is inferred by the history of "dry taps" on attempted sternal marrow aspiration. Absence at any time in her course of a demonstrated excess of any myeloid element in the peripheral blood handicaps further classification.

It seems best to draw no further conclusion from this case than unusually long survival of myeloid metaplasia, accompanied by an absorptive defect for cyanocobalamin. Though a characteristic reticulocyte response followed administration of vitamin B_{12}, and the patient had a smooth tongue and gastric achlorhydria, the diagnosis of pernicious anemia must be considered inconclusive. Profound macrocytic anemia was not observed and the patient's symptomatic response under

therapy, while impressive, might also be ascribed to cholecystectomy.

Discussion. Several published descriptions exist of pernicious anemia occurring in the same individual as did polycythemia vera:

ERYTHREMIA FOLLOWING PERNICIOUS ANEMIA. In several earlier reports of this sequence, the pernicious anemia appears to have been correctly diagnosed, but evidence for erythremia is unimpressive. Patients described by Goldstein[14], Adams[1], Skouby[29] and Weil and Boic[34] manifested red blood cell and hemoglobin levels while under therapy for pernicious anemia, which were slightly beyond accepted upper normal limits. However, other features of myeloproliferation usually seen in erythremia, that is, splenomegaly, leukemoid shift of circulating neutrophils, and increased platelets, were not described.

In at least one instance (Weil and Boic[34]) the authors were concerned that liver therapy might have overstimulated the marrow of their pernicious anemia patients, resulting in polycythemia. Accumulated experience with liver, folic acid and cyanocobalamin therapy over the past 30 years would seem to nullify such a concept. Reisner and Weiner[24], experimentally employing massive doses of vitamin B_{12} did not observe erythrocytosis beyond physiologic levels, and found the excess cyanocobalamin to be excreted in the urine in several subjects with pernicious anemia. Minot and Murphy[21] in their original report of the efficacy of oral liver therapy noted a few instances in which the red blood

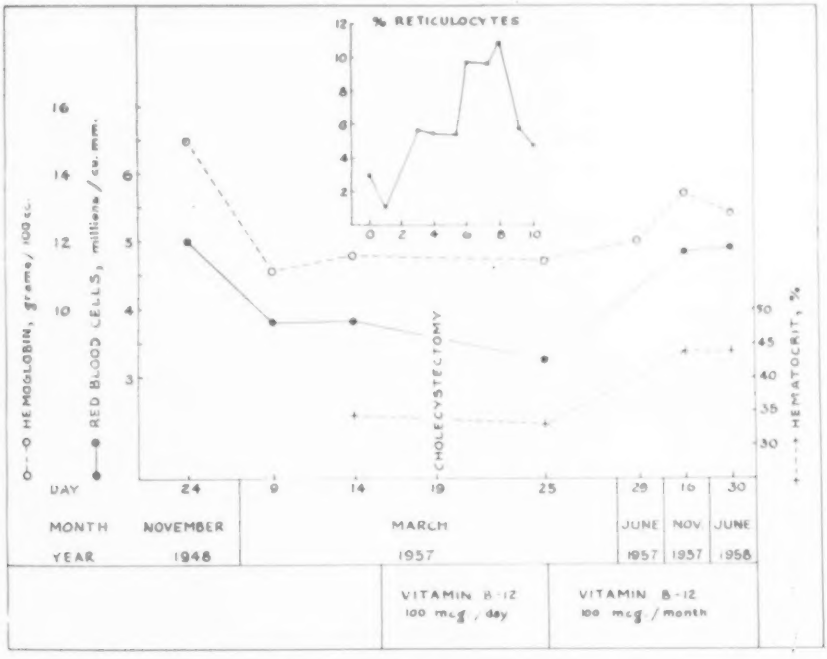

Fig. 3.—Erythrocyte, hematocrit and hemoglobin levels for Case 3.

cell count rose above 6,000,000 per c.mm. It seems reasonable to believe that in uncomplicated, fully treated pernicious anemia high red blood cell and hemoglobin values may be encountered with about the same frequency that the accepted "upper limit of normal" is violated in persons without hematologic disorders.

In two other individual case reports (Birnie[4], Ferrary[10]) red cell counts in excess of 10,000,000 and hemoglobin levels about 130% occurred in treated pernicious anemia patients. Here again no additional myeloproliferative phenomena are recorded, though in the absence of significant cardiopulmonary disease, erythrocytosis of this degree implies that erythremia truly may have been present in these instances.

Hinz's recent report[16] embodies adequate evidence to support the diagnosis of classical polycythemia vera in a patient who had been under treatment for some years for pernicious anemia. Death followed a cerebral hemorrhage attributed to the polycythemia. Zarafonetis, Overman and Molthan[38], in 1957, described an unprecedented sequence, also with fatal terminus, in which a patient manifested over a 5-year period pernicious anemia, erythremia and acute myelocytic leukemia, the last confirmed at necropsy.

Of exceptional interest is the experience with a patient first reported in 1952 by Galt, Hunter and Hill[13]. This elderly woman had originally presented classical clinical and laboratory features of pernicious anemia. Five months after initiation of parenteral liver therapy the spleen was noted to be palpable for the first time. Two months later red cell count was determined as 12,000,000 per c.mm. and hemoglobin as 24 gm.%. These high indices were lowered approximately to normal following phlebotomy and remained so for the next few months. It then became apparent that any single dose of more than 10 μg. of cyanocobalamin produced excessive hematopoiesis. The patient was unable to visit the author's clinic sufficiently often to determine whether or not a dosage schedule might be established which would provide "physiologic" marrow activity; as a consequence, hemoglobin determinations fluctuated between 6 and 19 gm., depending on the frequence of her visits and the vitamin B_{12} dose which was employed (Galt[12]). The patient was nevertheless living and in fair health after 6 years of coexisting pernicious anemia and erythremia when, in June, 1958, she consulted a different physician, unfamiliar with her unique hematologic problem. He apparently administered a considerable amount of cyanocobalamin, with a consequent erythrocyte level of 9,030,000 per c.mm. After removal by phlebotomy of 1500 ml. of blood, red cell count was 7,200,000 per c.mm. and the hemoglobin 14.6 gm.%. Galt also states that total white blood cell count rose to between 40,000 and 47,000 per c.mm. during this episode; previously, with very small doses of cyanocobalamin, it had remained in a range from 7,000 to 11,000 per c. mm.

A patient reported by Birnie[4] apparently ran a somewhat similar course, though details are lacking. After some initial wide variation between anemia and erythrocytosis, the author states that very small amounts of liver served to maintain erythrocyte levels within a reasonably physiologic range.

PERNICIOUS ANEMIA FOLLOWING ERYTHREMIA. Evolution of classical polycythemia vera into an anemic phase has been noted by a number of authors (Beattie and Withy[3], Block and Jacobson[5], Korst, Clatanoff and Schilling[19], Rosenthal and Basson[25], Wyatt and Sommers[37]). The anemia is often profound, immature forms of the white and red cell series are noted in circu-

lating blood, and myelofibrosis may usually be demonstrated. Prognosis in such "spent" erythremia is poor.

Appearance of anemia in a patient previously erythremic ordinarily implies either blood loss or onset of the "spent" phase. A review of the literature elicited 3 case reports, however, in which the authors inferred superimposition of pernicious anemia under such circumstances.

In a case reported by Freund in 1919[11], anemia became apparent rather abruptly after several years of polycythemic erythrocyte indices. He describes the smear of peripheral blood as consistent with pernicious anemia. However, as Wintrobe[35] points out, it is difficult to distinguish, from red cell morphology alone, pernicious anemia from the anemia that characterizes the "spent" phase of erythremia. Freund's patient succumbed to the anemia, a logical sequence for either disease at this time, prior to the discovery of an effective therapy for pernicious anemia.

Avery[2] in 1930 presented the case of a man with polycythemia who eventually became anemic. The patient did not respond to the oral administration of liver, and this together with other details of the clinical course suggest that here also was a case of "spent" erythremia.

Platt[23] in 1932 described an erythremic man who developed anemia after being bled 39 times over a 4-year period. The anemia in this case was markedly hypochromic and may have represented iron deficiency relative to the excessive blood-letting. There was improvement with oral liver, but concomitant with this therapy the patient also abstained from blood donation.

The foregoing cases, for reasons given, lack evidence to support the contention that pernicious anemia truly followed erythremia, and were the only reports found of such a sequence. That this possibility nevertheless exists is suggested by the circumstances in the first of our 3 cases.

MYELOCYTIC LEUKEMIA IN ASSOCIATION WITH PERNICIOUS ANEMIA. Studies of myelocyte behavior in uncomplicated pernicious anemia, have suggested that a "maturation arrest" may exist for leukocytes as well as for erythrocytes (Jones[18], Hirsch[17], Sharp Schleicher and Walter[27]). A characteristic marrow picture occurs, which may be modified by supervening acute infection (Strauss, Brokaw and Chapman[31]). In addition to the "macropolycyte," appearance in the peripheral blood of myelocytes, metamyelocytes and myeloblasts have been described in patients with pernicious anemia in relapse (Heck[15], Sharp, Schleicher and Walter[27]). The total number of myeloid leukocytes in peripheral blood is, however, consistently low. Morphology of leukocytes in both marrow and peripheral blood rapidly becomes normal under therapy.

In reviewing literature relevant to coexisting myelocytic leukemia and pernicious anemia, it is apparent that diagnosis of such a combination prior to the era of effective therapy for the latter disease was virtually impossible. It is not surprising that all 5 case reports (Sinok and Kohn[28], Sterne, Schire and Molle[30], Talley, Doherty and Shukers[32], Townsend[33], Woolley[36]) describing coexisting myelocytic leukemia and pernicious anemia appeared later than 1930, and with the leukemia developing during successful treatment of Addisonian anemia.

In these 5 case reports, interval between diagnosis of pernicious anemia and of leukemia ranged from 1½ to 10 years. We were interested in possible observations as to whether or not interruption of therapy of the pernicious anemia could influence the course of the leukemia. However, 4 of the 5

cases died within 3 months of the diagnosis of leukemia, and in the fifth, treatment (oral liver) was maintained for a year until death (Sinok and Kohn[28]).

Apart from mere freakishness, chance association in the same patient of an absorption defect for cyanocobalamin with abnormal myeloproliferation might contribute in some measure to understanding of various phenomena under the latter heading. The rarity of this association has hampered such observations.

From the first of our 3 cases, and from the case originally reported by Galt, Hunter and Hill[13], there is some reason to believe that the abnormal erythropoiesis of polycythemia vera cannot occur in the absence of cyanocobalamin. In 2 other valid case reports in the literature of coexisting Addisonian anemia and erythremia, the patients died too soon to allow relevant data. Our second case might have provided pertinent observations if allowed to go into pernicious anemia relapse, but her general disability including an existing neurologic defect militated against cessation or reduction of therapy.

The literature includes 5 case reports of coexisting pernicious anemia and myelocytic leukemia, with no inferences as to whether or not the leukemic process might be influenced by lack of cyanocobalamin. It seems probable that occasional further opportunity will arise for studying this association. Here, there is more justification for discontinuing cyanocobalamin therapy than in the erythremia-pernicious anemia association, to determine whether alteration of the myeloproliferative disorder could result.

Summary. Three cases are presented in which cyanocobalamin deficiency occurred in the same individual as did abnormal myeloproliferation. In one instance the patient clearly had polycythemia vera; of the other 2 patients, myeloproliferation was characterized in one by extraordinary platelet excess, and in the other by myeloid metaplasia and probable marrow fibrosis.

In the patient with polycythemia vera, the development of cyanocobalamin deficiency was noted to influence the course of the polycythemia. The implications of this observation are discussed, with a review of pertinent literature.

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SUMMARIO IN INTERLINGUA

Le Association de Carentia de Cyanocobalamina con Statos Myeloproliferative: Reporto de Tres Casos

Es reportate tres casos in que carentia de cyanocobalamina occurreva in le mesme individuo insimul con myeloproliferation anormal. In un del casos, le patiente habeva clarmente polycythemia ver. Quanto al altere duo casos, in un de illos le myeloproliferation esseva characterisate per un excesso extraordinari de plachettas e in le altere per metaplasia myeloide e probabile fibrose medullari.

In le patiente con polycythemia ver, il esseva notate que le disveloppamento del carentia de cyanocobalamina influentiava le curso del polycythemia. Le signification de iste observation es discutite. Un revista del pertinente litteratura es presentate.

IRON ENZYMES IN IRON DEFICIENCY: VII. OXYGEN CONSUMPTION MEASUREMENTS IN IRON-DEFICIENT SUBJECTS

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In earlier papers of this series, it has been demonstrated that not only hemoglobin, but also certain iron enzymes are depleted in iron-deficient experimental animals[1-5]. Heme enzymes play an important role in intracellular oxidations. Therefore, it seemed possible that decrease in the concentration of such vital iron enzymes as cytochrome C and cytochrome oxidase might result in a decreased rate of oxygen utilization by iron-deficient tissues. Preliminary experiments carried out on rat kidney slices failed to show any diminution in oxygen uptake when the tissue slices were incubated with glucose even when cytochrome C concentrations were markedly lowered (Necheles and Beutler[8]). However, under these conditions only minimal demands are placed on the enzymes of electron transport; it appeared possible that deficiencies in this system might become apparent only when the rate of oxidation was increased. In this study, therefore, we have measured the oxygen consumption of 4 iron-deficient subjects before, during and after moderately strenuous exercise, in order to determine whether iron therapy resulted in a decreased oxygen debt. Concurrent measurements of cardiac rate were also carried out on 3 of the subjects studied.

Materials and Methods. Four volunteer subjects suffering from varying degrees of iron deficiency were studied. Pertinent clinical data are given in Table 1. The methods for estimating hemoglobin, red cell count, hematocrit, plasma iron and iron-binding capacity have been described previously (Beutler, Robson and Buttenwisser[6]).

Each patient, in the fasting state, was allowed to rest in a reclining position for 3 or more minutes before any measurements were made. The subject then stood on the motionless treadmill and, after a 3-minute equilibration period of breathing through the apparatus, oxygen consumption measurements were made for 2 consecutive 3-minute periods. The treadmill was then set in motion at 3½ miles per hour, 0° grade, and the patient was exercised for 6 minutes, oxygen consumption being measured during the last 3 minutes of exercise. Oxygen consumption was also measured during the succeeding two 3-minute periods. Room temperature throughout the study was regulated at 24° to 25° C. by air conditioning. Subjects breathed room air through a one-way plastic valve. The ex-

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pired air was collected into Douglas bags. Oxygen concentration was determined by the Haldane apparatus or by the use of a Pauling oxygen meter, standardized daily against the Haldane method (Peters and Van Slyke[10]). Expired gas volumes and oxygen consumption have been calculated at standard conditions. Since each individual served as her own control, oxygen consumption has been presented merely as the milliliters of oxygen consumed per minute. Calculations based on body mass or surface area may be made from the additional data given in Table 1. Cardiac rate was determined from the electrocardiographic tracing obtained from a precordial electrode.

Results. The results of the studies are presented in Figs. 1 through 7. It is apparent that even before treatment with iron was instituted, there was a gradual decrease in oxygen consumption during exercise in all of the patients studied. This is believed to represent a "learning" process, and may be attributed to the patients' increasing familiarity with the treadmill and with the measuring procedures employed, resulting in gradually increasing efficiency in the work performed. In sub-

TABLE 1.—CLINICAL DATA

Subject	*Race*	*Sex*	*Age*	*Height (inches)*	*Weight (lb.)*	*Hemoglobin (gm.%)*	*HCT. (%)*	*RBC (10^6)*	*Plasma Iron (µg./100 ml.)*	*UIBC* (µg./100 ml.)*
D.C.	W	F	33	62½	108	12.0	38	3.60	65	215
D.G.	N	F	46	66	181	6.9	26	2.95	7	409
H.K.	W	F	44	68	158	12.7	40	3.91	44	430
J.K.	W	F	49	63	121	8.2	30	4.00	15	630

*Serum unsaturated iron-binding capacity.

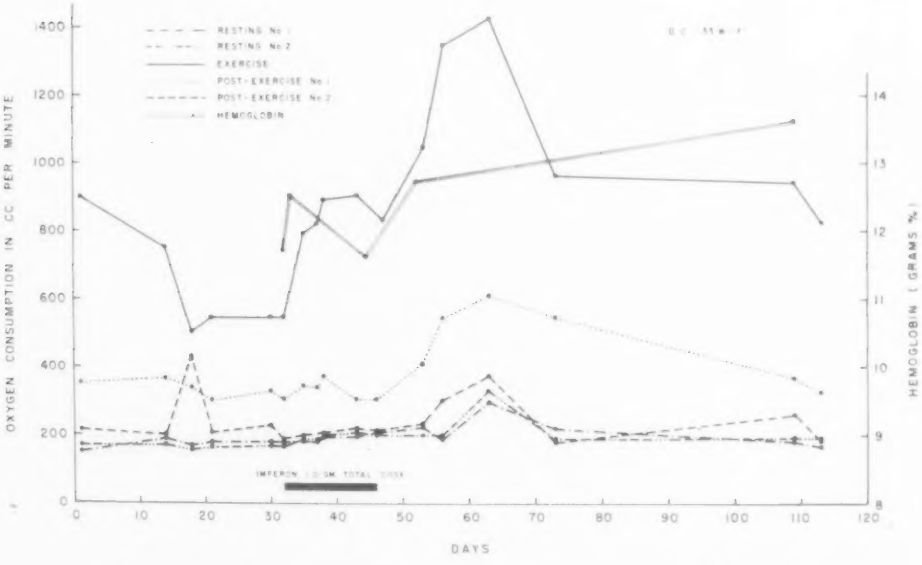

Fig. 1.—The effect of exercise on the oxygen consumption of subject D.C. before and during iron therapy.

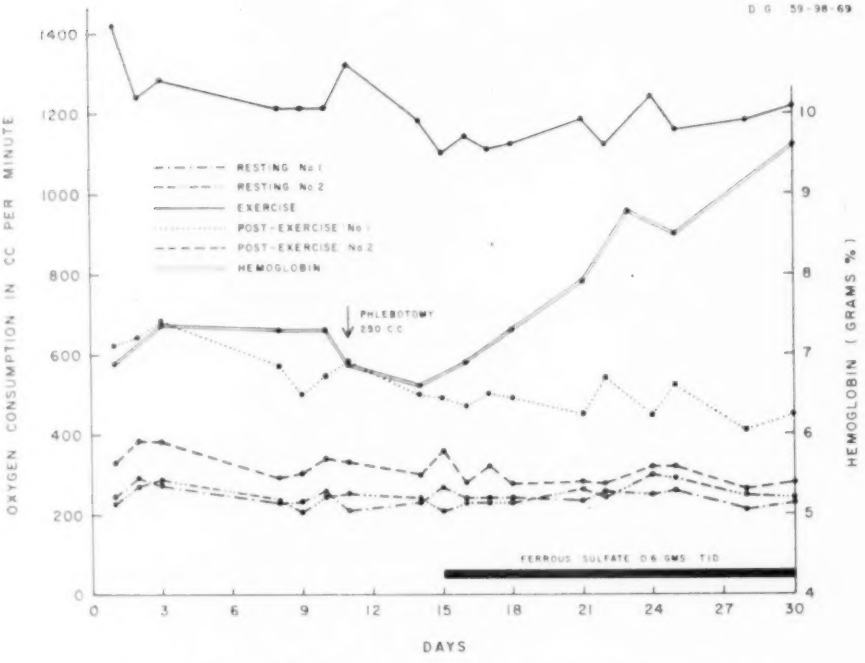

Fig. 2.—The effect of exercise on the oxygen consumption of subject D.G. before and during iron therapy.

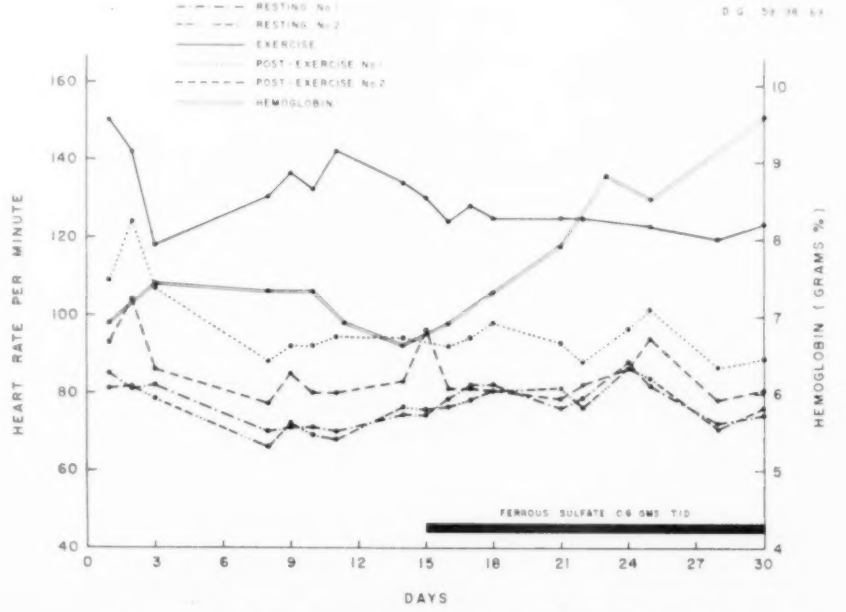

Fig. 3.—The effect of exercise on the heart rate of subject D.G. before and during iron therapy.

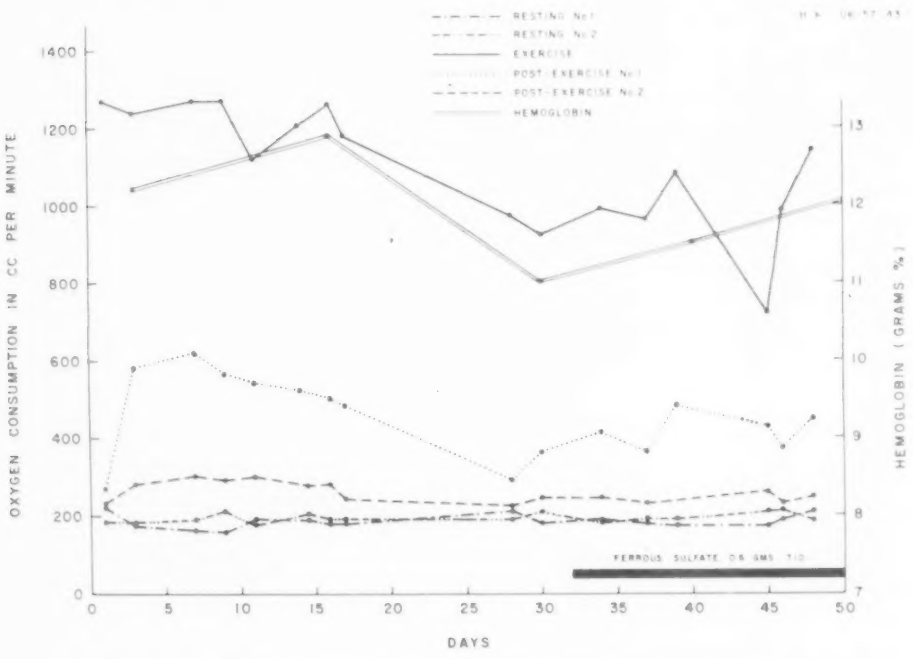

Fig. 4.—The effect of exercise on the oxygen consumption of subject H.K. before and during iron therapy.

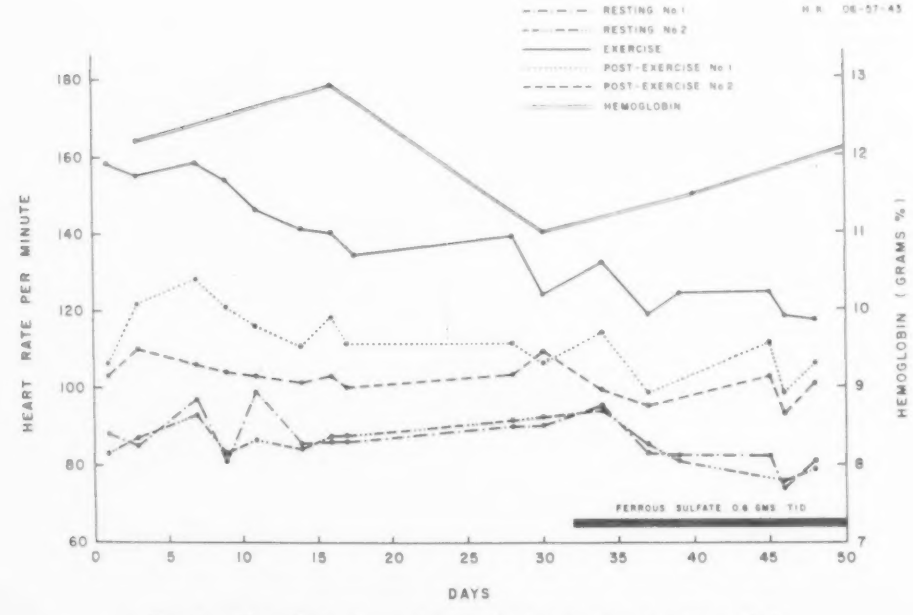

Fig. 5.—The effect of exercise on the heart rate of subject H.K. before and during iron therapy.

ject D.C., there was a marked increase in oxygen consumption during exercise following the institution of iron therapy. However, there was no apparent decrease in the subject's oxygen debt; oxygen consumption after the cessation of exercise was also increased. No such change was observed in any of the other subjects following treatment.

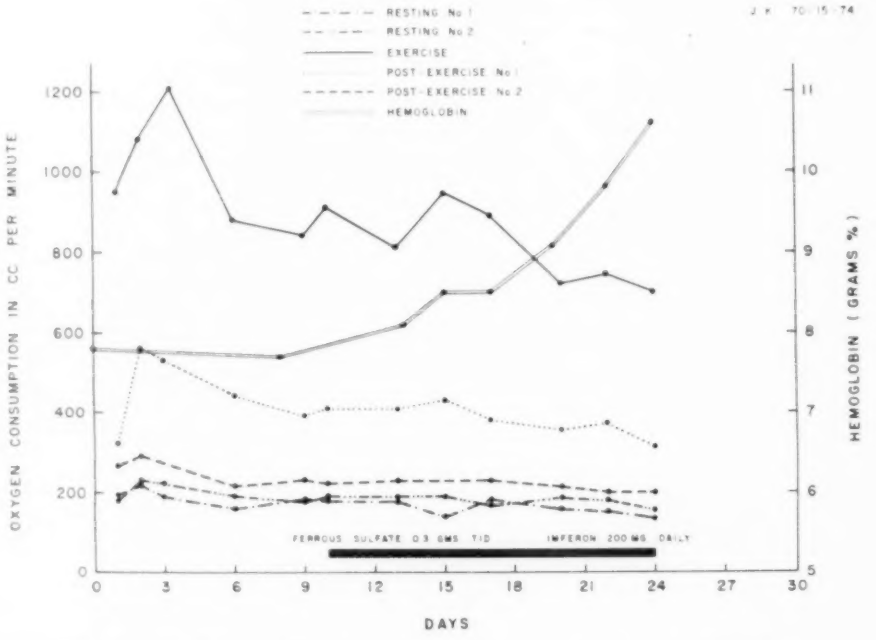

Fig. 6.—The effect of exercise on the oxygen consumption of subject J.K. before and during iron therapy.

Rather, there was a continued decrease in oxygen consumption, a continuation of the trend established before therapy was instituted.

No consistent effect of treatment on the cardiac rate was observed in any of the subjects studied.

Discussion. We have suggested previously that the symptoms of iron-deficiency anemia might be due to deficiencies in intracellular iron-containing enzymes, and have demonstrated that in experimental animals the concentration of some of the enzymes important in intracellular oxidations are decreased in iron-deficient experimental animals (Beutler[1,2], Beutler and Blaisdell[4]). Our earlier studies have shed no light on the problem of whether the decrease in tissue enzymes observed was sufficient to limit the rate of oxidative metabolism *in vivo*. If a well-marked, widespread insufficiency in the rate at which oxidative reactions could take place occurred in iron-deficient subjects, one would expect the oxygen consumption of an iron-deficient subject to be less than that of a normal subject during exercise, and one would expect an abnormally large oxygen debt to be incurred. When an iron-deficient subject was treated with iron one might therefore

expect the oxygen consumption during exercise to increase and the oxygen debt to decrease. With the relatively crude methods used, no such change was observed. It is of especial interest that even when the hemoglobin level of these subjects had increased considerably, no change in oxygen debt or even of cardiac rate was observed.

Iron plays its vital roles in the body economy in a variety of ways; participation in oxygen transport, either as hemoglobin in the blood stream or in intracellular iron enzymes, is only one of these functions. Iron has other functions not concerned directly wth oxygen transport. It is probably a co-factor in aconitase activity (Dickman

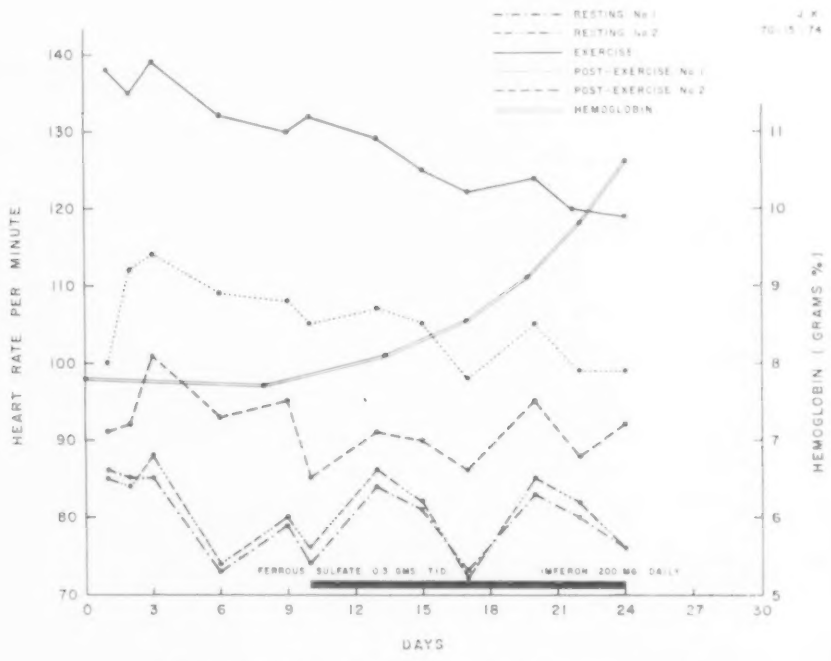

Fig. 7.—The effect of exercise on the heart rate of J.K. before and during iron therapy.

It has often been alleged that the symptoms in iron-deficiency anemia are due to the decreased oxygen-carrying capacity of the blood. The results of this study demonstrate how little effect the lowering of oxygen-carrying capacity of the blood has on oxygen consumption and oxygen debt incurred by the organism during exercise. The compensatory mechanisms of the body are apparently exceedingly efficient in this respect.

and Cloutier[7]). It is a co-factor in at least one transamination reaction (Patwardhan[9]) and there is experimental evidence that in microorganisms it may play an important role in ribonucleic acid synthesis (Winder and Denneny[11]). That iron may play an important synthetic role in man is also suggested clinically by the marked changes in fingernail growth which occur in the iron-deficient subject.

While this study does not rule out

the possibility that the symptoms of iron deficiency may be due to minor or localized changes in intracellular oxidations, it serves to emphasize that the manner in which iron deficiency produces symptoms is probably quite subtle and remains to be elucidated.

Summary. Oxygen consumption and heart rate have been studied before, during and after standardized periods of exercise in 4 iron-deficient subjects, before and during treatment with iron. No consistent change in oxygen consumption was observed. Treatment did not significantly alter the cardiac rate. These findings suggest that the symptoms of iron deficiency may arise from a mechanism other than a generalized defect in oxygen transport.

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SUMMARIO IN INTERLINGUA

Enzymas a Ferro in Carentia de Ferro: VII. Mesurationes del Consumption de Oxygeno in Subjectos con Carentia de Ferro

Le consumption de oxygeno e le frequentia cardiac esseva studiateante, durante, e post periodos standardisate de exercitio in 4 subjectos con carentia de ferro, e iste studios esseva repetite ante e durante le tractamento con ferro. Nulle uniforme alteration del consumption de oxygeno esseva observate. Le tractamento non alterava le frequentia cardiac de maniera significative. Iste constatationes pare provar que le symptomas de carentia de ferro resulta de un mechanismo altere que un defecto generalisate in le transporto de oxygeno.

PROGRESS
OF
MEDICAL SCIENCE

GYNECOLOGY AND OBSTETRICS

UNDER THE CHARGE OF

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THE STUDY OF FETAL PROBLEMS WITH ELECTRONIC METHODS

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PERINATAL fetal and infantile losses account for more than 160,000 deaths annually in the United States. These constitute approximately 10% of deaths from all causes and at all ages[2].

About half of all perinatal deaths are encountered before or during labor, and most of the remainder during the first 48 hours of the neo-natal period. In over two-thirds of the fatalities, the fetuses and infants are of a gestational age so inadequate as to constitute, in itself, the major problem. Unfortunately, at the present time the causes of premature birth are understood in less than half of the observed cases. Any substantial reduction of overall mortality rates, therefore, must await the acquisition of more fundamental knowledge concerning the etiology of premature birth, and the utilization of this knowledge by obstetricians and public health agencies.

A more favorable opportunity for

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early improvement would appear available in attempting to reduce the number of fetal and infantile deaths that occur in and around labor at term. The causes of such deaths can usually be identified. In many instances the known dangers can be forestalled, or their impact upon the fetus cushioned, by appropriate obstetric care and skill. Moreover, there is generally a warning of impending difficulty manifested in the development of certain clinical signs of hypoxial or asphyxial "fetal distress." In term fetuses these signs are more significant, and more readily recognized as such, than in premature fetuses. Failure to detect them may cause the fetus to be lost before its birth, to be born in such a depressed condition as to succumb shortly thereafter, or to survive only as a palsied, spastic or mentally retarded individual.

Clinically, fetal asphyxial distress is recognized by its effects upon fetal cardiac action. During late pregnancy and the first stage of labor at term, the fetal heart normally beats at an average rate of about 140 strokes per minute. During the second stage of labor, this rate may not infrequently be depressed to 90 or 100 beats for brief intervals following each uterine contraction. This phenomenon, in cephalic presentations, is not believed to signify more than the effects of physiologic pressures intermittently exerted upon the advancing fetal head. On the other hand, depressions of rate to levels lower than 80 beats, or elevations above 180, particularly if persistent, suggest a state of significant fetal hypoxia during any stage of labor or period of pregnancy in which they may be encountered. The development of these alterations of rate may be associated with irregularities of cardiac rhythm, release of meconium from the fetal rectum, and unusually vigorous general fetal activity.

The traditional method of auscultation of the fetal heart beat with the use of a stethoscope applied to the mother's abdomen, first described by Mayor[28] in 1818 and by deKegaradec[12] in 1821, leaves something to be desired as a means of detecting early difficulties. Transient fluctuations of cardiac rate frequently go unnoticed, and there is a wide margin of human error in evaluating what is heard. Throughout the hours usually occupied by the dilatation of the os uteri in the first stage of labor, that is, the period during which most of the significant disturbances of fetal oxygenation occur, the availability of a practical mechanical device for the constant monitoring of fetal cardiac action would undoubtedly serve to improve fetal well being and salvage.

As a result of advances in the field of electronics over the past 30 years, efforts have been made to devise and test equipment for the above purpose. Such efforts have been accelerated during the past decade, and a review of some of this work will be considered here.

FETAL PHONOCARDIOGRAPHY. The first microphonic reproduction of fetal heart sounds was achieved by Falls and Rockwood[14] in 1923, using instruments designed by Abbott[1] a year earlier. The clinical applicability of this equipment was limited, however, by excessive "pickup" of extraneous noises and acoustic feedback "howls."

In 1937, Matthews[27] succeeded in designing an instrument which eliminated most of these difficulties. The apparatus proved to be so complicated, however, that even the designer felt it to be impracticable for hospital use. Its main value was that the heart beat could be amplified and broadcast for the information of supervisory personnel when not in immediate attendance at the mother's bedside. A practical,

small fetal phonostethoscope has subsequently been designed by Palmrich[30].

In 1938, DeCosta[11] devised a photostethoscope by which a microphonic pickup of fetal heart tones could be made to produce pulsations of light in a small neon lamp. This eliminated acoustic feedback, but required continuous supervision and rather tiring observation and counting.

Efforts to produce a permanent graphic record of fetal heart sounds led to the development of an electrostethograph in 1935 by Lockhart in association with Bierring *et al.*[7]. With the use of this equipment, photographic patterns of heart sounds were made possible in approximately 60% of mothers examined during late pregnancy and labor. Subsequently, Smith and Hervert[32,33] made a number of observations of fetal heart sounds, using Lockhart's equipment, combined with simultaneous electrocardiograms of both mother and fetus. Similar studies were made by Paley and Krell[29]. A. L. Smith[32] made the first successful stethograph and disc-recording of a twin pregnancy; he also demonstrated the viability of both fetuses of a twin pregnancy in an instance where the death of one had been suspected clinically.

The further development of fetal phonocardiography was delayed for a time by a temporary lack of progress in acoustical research. Renewed effort, however, has apparently been kindled by the remarkable technical advances that have lately been made in the field of high-fidelity sound reproduction. Currently, attention is being directed to devising apparatus for picking up the fetal heart tones with a microphone, filtering them, amplifying the signals, and recording them upon graph paper.

The problem of eliminating extraneous noises remains. Gunn and Wood[16] showed that the basic frequency of fetal heart tones is 16 cycles per second (CPS), with the usual harmonics also present (that is, 32 CPS, 64 CPS, 128 CPS). Hence, audio filters attuned to these frequencies should prove helpful in eliminating unwanted "noise." The first fetal cardiotachometers were described in 1957 simultaneously by D. H. Smith[34] and by Corner and Stran[9]. Smith successfully reduced much interfering sound by filtering all impulses outside of the range of 60 to 80 CPS. Another type of phonocardiograph was described at about the same time by Dewhurst and Mainland[13]. In this case, advantage was taken of the sensitivity of the apparatus for picking up sound frequencies in a range that is inaudible to the human ear, and then filtering out all higher frequencies, that is, limiting recordings to the range of 16 to 26 CPS. The value of audio filtration was further shown by Sawyer[31], who found excellent beat-patterns in the range between 30 and 80 CPS. Filtration that excludes impulses in the range above 128 CPS will serve to exclude much highpitched noise caused by rustling of bedsheets, bowel sounds and the placental souffle.

From the standpoint of sparing apprehensive mothers the experience of hearing what is going on, the ideal cardiotachometer should register the fetal heart rates silently and continuously on a dial. The latter should be calibrated in such a manner as to trigger a warning light whenever the rate drops below 100 or rises above 180 beats per minute. The engineering problems involved in designing such equipment have already been explored by D. H. Smith, who developed a fetal heart rate meter in 1957, but who has not yet published the details of his clinical studies. Tolles[40] designed a cardiotachometer in which an amplitude trigger-circuit is employed. The employment of this principle is at-

tended by difficulties in adjusting sensitivity levels, and by variations of amplitude in the heart sounds themselves, as well as of extraneous sounds. Sawyer has overcome many of these difficulties by designing a special heartbeat detector circuit which picks up the filtered impulses and triggers a tachometer. His equipment measures the rate by beat-to-beat intervals, and indicates it on a dial. A graphic record or taped recording can be made for further study. Continuous monitoring of the equipment with an oscilloscope is necessary, however, in order to maintain relatively constant signal-sensitivity levels.

Studies of pregnant women with the use of Tolles' apparatus have been published by Hellman *et al.*[17], who were able to obtain useful recordings in about half of the subjects examined. The more refined equipment built by Sawyer produced adequate records in 10 out of 13 patients. Such studies will doubtless be extended in future. On the other hand, much remains to be done in the technical developmental area that has been commanding the principal attention of investigators up to the present time.

FETAL ELECTROCARDIOGRAPHY. The identification of a fetal electrocardiographic complex was first reported by Cremer[10] in 1906, having been noted by him during the course of obtaining the mother's electrocardiogram (EKG). Since that time various efforts have been made to obtain improved fetal EKG tracings[3,4,15,18,22,24,26,37,39]. These have entailed the use of preamplifiers for magnifying the pick-up of the weak fetal cardiac electropotentials, as located with the use of electrodes suitably placed over the maternal abdomen, or in the maternal vagina, rectum or bladder[16,21,35].

Inasmuch as the maternal EKG is simultaneously obtained and recorded, and because it registers pulsations of greater amplitude than those of fetal origin, various means of damping or cancelling out this "interference" have been explored. On the whole, however, the maternal component in the average tracing does not seriously interfere with the recognition and interpretation of the fetal EKG. More troublesome artefacts are apt to be encountered if the electrode "leads" are not well located or firmly secured, or if the electrical pick-up is altered by maternal intestinal peristaltic activity or somatic muscular tremor. The recordings may also be modified by variations in the thickness of the overlying abdominal walls, by an interposed placenta, the amniotic fluid and even the vernix caseosa[4,37,38].

The factors noted above reduce the fetal EKG tracing to a simple QRS wave complex, since the P and T waves are usually obscured. Nevertheless, alterations of the QRS wave complex and ST segment have been noted, in some instances as early as the second trimester of pregnancy[24]. In such cases a cardiac anomaly may later be found at the time of the child's birth. In general, however, the fetal EKG is mainly of use for studying the cardiac rate and rhythm.

For this purpose useful tracings can occasionally be demonstrated as early as the 12th week of pregnancy[23]. It is not infrequently possible to obtain a fetal EKG 2 to 6 weeks before heart tones can be detected by auscultation. From the second trimester to term, positive results can be secured in 85% of patients, and during the last trimester in practically all cases[4].

The most extensive recent studies of fetal heart rate patterns with the use of the EKG have been carried out by Hon[18,19,20,21,22]. By monitoring the rates to be observed during the course of labor, he has felt it possible to rec-

ognize correlations between them and certain normal and pathologic phenomena. He has defined fetal bradycardia as being physiologic or pathologic. The former type is essentially that which has already been referred to in an introductory paragraph of this review as occurring during the second stage of labor; according to Hon, it may be observed also during the late, or "accelerating," phase of the first stage of labor. The pathologic type may occur in two patterns. One is a bradycardia which sets in shortly following the onset of a uterine contraction and persists briefly beyond the end of the contraction. It is thought to develop primarily as a reflex response to fetal hemodynamic changes brought about by compression of the umbilical cord; it can be evoked in instances of occult prolapse by manual pressures exerted simultaneously upon the fundus of the resting uterus and upon the fetal head as it sits against the brim of the maternal pelvic inlet. The other pattern of pathologic bradycardia is one that develops approximately 20 seconds before the end of a uterine contraction, lasts beyond the end of the contraction, and then disappears only very slowly. This pattern has been observed principally in cases of maternal arterial hypotension. It is also seen in instances where the uterine contractions are exceptionally strong or spastic, and where the uterus fails to relax sufficiently between such contractions. This pattern is attributed to fetal hypoxia developing as a result of lowered oxygen tensions in the intervillous placental sinusoids.

The fact that the fetal EKG in its present stage of development rarely reveals significant alterations of QRS wave pattern, and that it promises to be of use to the obstetrician mainly as a reliable and very sensitive method of monitoring fetal cardiac rate and rhythm, does not limit its potential solely to the detection and study of hypoxial fetal distress. It offers promise of being helpful, or has already proven so, in the differential diagnosis of pregnancy from such conditions as maternal obesity, fibroids, abdominal cysts, pseudocyesis and hydatidiform mole; in the determination of fetal viability under a variety of complicating circumstances; and in the early detection of multiple-fetus pregnancy. Its use for the latter purpose in circumstances of clinical doubt could supplant present dependence upon roentgenography. Thus, Larks[23] correctly diagnosed 9 sets of twins, one set of triplets and one set of quadruplets from fetal EKG's taken in midpregnancy. Finally, since the polarity of the fetal QRS wave deflections varies with fetal polarity and lie, it can be utilized as an adjunct in the diagnosis of malpresentations[3,4,37].

FETAL ELECTROENCEPHALOGRAPHY. Brief reference only will be made here to the work of Lindsley[25], and of Bernstine *et al.*[4,5,6,8], who have given much attention to the difficult task of attempting to develop fetal electroencephalograms (EEG) as a means of evaluating fetal well-being. With the use of special electrodes applied over the abdomens or in the vaginas of expectant mothers, they have observed electropotential phenomena which could not be explained entirely as originating in the maternal tissues or fetal heart. They have reported that the tracings obtained have resembled recordings taken directly from the craniums of infants immediately after birth. In a study of 32 women by Bernstine and Borkowski[4], the recordings obtained from only 4 of the subjects failed to show significant activity, and in 2 of these instances the fetuses were known to have been dead *in utero*. Although Bernstine and Borkow-

ski[5] have now extended their studies to include examinations of 75 women, and have felt encouraged to believe that there is hope of eventually demonstrating derangements of fetal cerebral metabolism, the problems that remain to be looked into by continuing effort would appear to be formidable.

COMMENT. The electronic devices that have thus far been developed for the study of fetal problems remain essentially investigative rather than service equipment. For their purposes, they are remarkably sensitive and reliable. But the areas of exploration to which they lend themselves in the study of fetal hypoxia will not in themselves suffice for delineating all the parameters of this problem. To the extent that further work is correlated with data simultaneously obtained by other investigative methods[36], it is to be hoped that not only will a more complete understanding of the relations between fetal distress and its cardiac manifestations be attained, but that the application of this knowledge to clinical work will result in substantial gains in fetal well-being and salvage.

Summary. Through the use of sensitive electronic equipment, important advances are being made in the study of the condition of the fetus *in utero*. In cases of suspected fetal death, the question of viability may be examined and determined with fetal electrocardiograms or phonograms. The same devices may be utilized to determine the number and viability of fetuses present in a suspected multiple pregnancy, without incurring the risks attending the current use of roentgenography for this purpose. The fetal electrocardiographic QRS wave complex varies in polarity with the polarity of the fetal lie *in utero*, and can therefore be utilized to establish or confirm the presence and nature of suspected malpresentations. The most important potential for the usefulness of these devices, however, appears to lie in their application to the study of acute hypoxial fetal distress during labor. With the correlation of data now being accumulated, precise parameters of true fetal distress are being delineated. Electronic monitoring of fetal cardiac activity before and during labor may eventually serve to reduce perinatal fetal loss and morbidity through the early detection of correctable factors.

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RENAL MANIFESTATIONS OF SICKLE CELL DISEASE: A REVIEW*†

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SINCE Herrick[18] reported the first patient with sickle cell anemia in 1910, genitourinary symptoms, kidney pathologic findings and renal dysfunction have been frequently reported in subjects with sickle cell disease[40]. In the past decade particularly this subject has been receiving increasing interest.

Among the first 20 cases of sickle cell disease in the literature, those reported by Herrick[18], Washburn[40], Mason[31], Sydenstricker[38], and Huck[21] mentioned casts[18,21,31,38,40], leukocytes[40], albumin[18,21,31] and low specific gravity values of urine[18,21,38]. Since then, a multitude of morphologic changes in the kidneys, recurrent unilateral gross hematuria, hyposthenuria, altered renal hemodynamics, pyelonephritis and nephrotic changes have been reported in patients with sickle cell disease.

MORPHOLOGIC CHANGES IN THE KIDNEY. Sydenstricker, who reported the first necropsy findings in a 5-year-old Negro boy with sickle cell anemia, found the kidneys to be pale, increased in size, and scarred[38]. Microscopically the glomeruli were strikingly prominent and distended by blood. Pericapsular fibrosis of some glomeruli, cloudy swelling, pigmentation and necrotic changes of tubular cells were also reported. More recent reports confirm these findings and include others. Bernstein and Whitten[5] interpret the prominence of the glomeruli of the juxta medullary region as evidence of increase in vascularity and cellularity

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†Any subject whose red cells contain 'S' hemoglobin will be considered as having sickle cell disease. This designation, therefore, includes individuals with sickle cell trait, 'S' C disease, sickle cell thalassemia, as well as patients with 'pure' sickle cell anemia.

rather than simple passive congestion. In addition, they found focal glomerular hemorrhages with exudation of blood in Bowman's space and around the tubules. This seemed to be the first step resulting ultimately in fibrosis and scarring of glomeruli. Active tubular necrosis, regeneration and pigmentation were also reported, as were alterations in the medullary papillae with high frequency. Edema, telangiectasia, increased amount of connective tissue, a decrease in the number of tubules, constricted epithelial nests, tubular retention cysts, and frank loss of substance with blunting of the pyramides were reported.

Patients with sickle cell trait sometimes show comparable renal pathologic changes[14,29,33,39]. However, the incidence of pathologic findings in sickle cell trait cannot be determined with certainty since necropsy and biopsy findings of the kidneys of symptomless sickle cell trait subjects are rarely reported. In 23 patients in whom one kidney was removed because of gross unilateral hematuria, Mostafi *et al.*[33], and Lund *et al.*[29] found the kidneys normal in 5 instances. The diagnosis of sickle cell trait in these patients was based on the clinical picture, the presence of positive sickling and absence of anemia. Electrophoretic identification of the amount of 'S' hemoglobin was not available. The kidneys in sickle cell trait have been reported to be congested and grossly show cortical, or medullary, or both cortical and medullary, and submucosal hemorrhages[14,29,33,39]. Congestion and hemorrhages in the renal pelvis and papillary necrosis have also been noted. Microscopically, the glomeruli are congested, and in a 35-year-old patient[39] who died in uremia, many glomeruli were diminished in size[29,33,39]. The peritubular capillaries show severe patchy stasis, most marked in the medulla, and extravasation of blood into peritubular areas and into tubules with separation of tubular epithelium from the basement membrane and desquamation of the epithelial cells[33]. A large number of patients had extravasation of blood in the area surrounding the collecting tubules. In small segments of the tubules, foci of degeneration were sharply demarcated from regions of regeneration[14,33].

Edema of the papillae, degenerative changes of the papillae and proliferation of the epithelium of collecting tubules are frequently seen[33].

Pyelonephritic changes of the kidneys of sickle cell anemia patients have also been reported[4,7,39].

GROSS HEMATURIA. Abel and Brown[1] and Fetter and Schnabel[12] were the first to report gross hematuria in sickle cell disease. Since then, many additional cases have been reported, some authors collecting as many as 22 cases[33]. Patients with gross unilateral hematuria have been subjected to nephrectomy in almost 40% of the reported cases[36]. Sharpe *et al.*[36] stress the need for conservative management since Lund *et al.*[29] and Arduino[2] report patients who, after nephrectomy, had recurrence of hematuria in the remaining kidney. They, as well as Crone *et al.*[8], managed their patients conservatively with satisfactory results. Occasionally the clinical picture and the roentgenographic findings of patients with hematuria and sickle cell disease may simulate renal neoplasm[1]. These patients will be saved from unnecessary nephrectomy if they are reexamined in a few days after the attack of hematuria. In one series, hematuria in sickle cell disease has been reported to occur in 3 out of 54 patients[16], and in another series, in 2 out of 142 patients[34]. Smith and Conley[37] found that of their 27 patients with sickle cell 'C' disease, 4 had hematuria

and conclude that these patients have more renal involvement than subjects with other 'S' hemoglobinopathies. Hematuria has been reported more frequently in sickle cell trait than in sickle cell anemia[8,13,14,17,29]. Sharpe *et al.*[36] reported that out of 6 patients with sickle cell 'C' disease, 3 had hematuria; that 2 out of 54 subjects with sickle cell trait had hematuria but that none of their 22 patients with sickle cell anemia had hematuria. Of 44 cases they collected from the literature in which the hemoglobinopathy had been identified electrophoretically, hematuria was reported in 15 patients with sickle cell 'C' disease, 28 patients with sickle cell trait and in one patient with sickle cell thalassemia. Berman and Tublin reported an 8-year-old Negro boy with the nephrotic syndrome and proven sickle cell anemia[4]. This patient had a history of hematuria. Hoefnagel also reported a case of sickle cell anemia with gross hematuria[20]. From these scattered reports one may conclude that hematuria occurs in sickle cell anemia but less commonly than in sickle cell trait.

Hematuria is unilateral and more frequently from the left kidney. Of 48 case reports collected from the literature[8,13,14,29,33,36] 36 had hematuria from the left kidney, 10 from the right kidney, and in 2 instances, the hematuria was bilateral[8,13].

Kidneys examined after nephrectomy may show no changes which account for the hematuria[29,33], slight changes such as submucosal hemorrhages[33] or extensive changes such as necrosis of the papillary areas[33].

TABLE 1.—RESULTS OF URINALYSIS IN 40 PATIENTS WITH SICKLE CELL DISEASE

	Sickle Cell Trait	*Sickle Cell Anemia*
Total number	27	13
Normal urinalysis	22	9
Abnormal urinalysis	5	4

Findings in Patients with Abnormal Urinalysis

Name	*Diagnosis*	*Protein*	*Leukocytes*	*Erythrocytes*	*Casts*
1. G.S.	S.C. Trait	1+	13-20/HPF	—	few
2. L.M.*	S.C. Trait	—	—	innumerable	—
3. B.W.†	S.C. Trait	1+	numerous	few	—
4. A.W.	S.C. Trait	—	6-10/HPF	few	—
5. D.J.	S.C. Trait	—	numerous	—	—
6. J.C.	S.C. Anemia	—	15-18/HPF	occasional	occas.
7. F.H.	S.C. Anemia	2+	—	—	occas.
8. R.H.	S.C. Anemia	trace	10-12/HPF	—	—
9. Z.V.	S.C. Anemia	trace	—	—	—

*Admitted with gross hematuria.
†Admitted with urinary tract infection.

The findings on routine examination of urine in patients with sickle cell anemia and in subjects with sickle cell trait are presented in Table 1. Abnormalities are noted in both trait and anemia patients.

HYPOSTHENURIA. Low specific gravity values of urine in sickle cell anemia patients were mentioned in the earliest reports of this disease, but urinary concentration tests were not performed[18,21,38].

In 1928, Joseph reported, in his series of 16 patients with sickle cell disease, one patient with arterial hypertension and with tendency to fixation of the specific gravity of urine[22]. The hyposthenuria and hypertension were reported to have cleared following transfusion. Mention was not made of the age of the patient or of the urinary specific gravity values. Higgins and Toone[19] also observed low urinary specific gravity values and Henderson[16] in his report of 54 patients with sickle cell disease found urinary specific gravities between 1.002 and 1.015. Margolies[30] and Leavell and MacIlwaine[26] in their reviews of sickle cell anemia made mention of the fact that hyposthenuria occurs but were not sure that this was a constant finding.

In 1953, McCrory *et al.*[32] and Kunz *et al.*[25] reported the first detailed investigations on the subject and established that the urinary concentration defect is not due to a deficiency of antidiuretic hormone. They suggested a renal etiology for the hyposthenuria.

In 1954, Zarafonetis *et al.*[44] reported that sickle cell trait subjects also failed to concentrate urine normally and postulated a genetic basis for the hyposthenuria. Two years later Zarafonetis *et al.*[43] reported that the urinary concentration defect is less pronounced in sickle cell trait than in sickle cell anemia. After 18 hours of water deprivation, the mean urinary concentration of 16 sickle cell anemia patients, ranging in age from 15 to 44 years, was 420 mOsm. per L. (range 320 to 551). Of 36 sickle cell trait subjects ranging in age from 13 to 57 years the mean urinary concentration maxima was 604 mOsm. per L. (range 413 to 980). Only 4 of these 36 subjects concentrated urine normally. They also mention that relatives of their subjects who did not have 'S' hemoglobin in their red cells concentrated urine normally. Keitel *et al.*[24] demonstrated that the hyposthenuria of young patients with sickle cell anemia was reversible after multiple transfusions with normal erythrocytes. Reversibility of hyposthenuria was less pronounced, or not present, in older patients. These authors postulated that renal damage due to intravascular sickling or to the presence of 'free' sickle cell hemoglobin may result in renal dysfunction and hyposthenuria. Levitt *et al.*[28] found that reduction in the glomerular filtration rate of 50% in 6 hydropenic sickle cell disease subjects produced an 8% to 20% fall in urine osmolarity. This is the same change which has been observed in normal control subjects. Since this eliminates the possibility of an ADH resistant impermeability to water in the area of the nephron where urine is concentrated, they hypothesize that subjects with 'S' hemoglobin are less capable of achieving a normal degree of medullary hypertonicity, probably due to the abnormal vascular architecture at this site. Actual measurements of the osmolar concentration in the medullary tissue of sickle cell anemia kidneys are not yet reported[42].

The 'free' water clearance has been reported by Keitel[23] as being normal, or supranormal, in his sickle cell anemia patients. Their ability to dilute urine is not impaired, or sometimes is greater than normal. The amount of free water reabsorbed during osmotic diuresis[41] is reported as being only slightly impaired in sickle cell anemia patients. This indicates that the renal tubules are able to reabsorb a basically normal amount of solute free water when the amount of osmotic work to be accomplished is small. Sickle cell anemia is the only known disease where a discrepancy between Tm^cH_2O and the maximal urine concentration exists.

Schlitt and Keitel[35], reporting on

urinary concentration maxima of subjects with sickle cell disease of different ages, found a statistically significant inverse relationship between the urinary concentration maxima and increasing age in sickle cell trait and sickle cell anemia. One can only speculate as to the basic nature of this change, possibly it is due to clinical or subclinical sickling, or both, or to the presence of 'free' S hemoglobin. At any rate, until one knows precisely how urine is concentrated in normal subjects, which is not the case at the present time, it seems idle to speculate excessively why hyposthenuria is present in sickle cell disease. It is noteworthy that hyposthenuria is much more common than anemia in sickle cell disease even though sickle cell disease is classified as a blood disorder.

RENAL HEMODYNAMICS. The first report concerning the glomerular filtration rate (GFR) in sickle cell disease subjects was by Calcagno *et al.*[6]. They found a low GFR in 5 patients ranging from 4 to 15 years of age. Henderson[16] reported the urea clearance as being normal in 2 patients, and Berman and Tublin found a normal creatinine clearance in a boy with sickle cell anemia and the nephrotic syndrome[4].

Etteldorf *et al.* found the GFR increased by 0% to 71% in 8 sickle cell anemia subjects who were 4 to 11 years of age[10]. The effective renal plasma flow was increased by 18 to 117% and the effective renal blood flow by 24 to 105%. In 1955, Etteldorf *et al.*[9] investigated young adults with sickle cell anemia and found that the GFR, the effective renal plasma flow and the Tm PAH were normal and supranormal. Between 20 to 30 years of age, however, these functions began to decrease and after 30 years of age, all renal functions were found to be depressed. The GFR:Tm PAH ratio decreased with age; this indicates a disproportionate decrease in the GFR. Reports of other authors[15,27,32,43] confirm these findings. Zarafonetis *et al.*[43] reported on the urea and creatinine clearance in 16 sickle cell anemia subjects ranging in age from 15 to 44 years of age, and 36 in sickle cell trait subjects ranging from 13 to 57 years. They found the means of the clearances higher in sickle cell anemia but the range was as wide as in sickle cell trait (see Table 2).

TABLE 2. *(Condensed from Zarafonetis)*

	Urea Clearance	*Creatinine Clearance*
Sickle cell anemia	121% (60-173)	103% (65-151)
Sickle cell trait	107% (27-175)	89% (51-150)

NEPHROTIC SYNDROME[1]. Three cases of the nephrotic syndrome in sickle cell disease subjects have thus far been described[3,4,11]. Berman[4] reported a poor response to treatment with corticotropin.

Summary. Renal involvement occurs early in the course of sickle cell disease.

A review of the pathologic findings of renal tissue is presented with an attempt to correlate the findings with the clinical features.

Hyposthenuria is the earliest functional disability, and is found in almost all patients with sickle cell anemia and in the majority of subjects with sickle cell trait. Possible mechanisms of development of hyposthenuria are presented but it is concluded that without definitive knowledge of how urine is concentrated in normal subjects, this problem is insolvable.

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Correlation of Structure and Function in the Human Eccrine Sweat Gland. RICHARD L. DOBSON, M.D. (Division of Dermatology, Department of Medicine, University of North Carolina School of Medicine. Chapel Hill, N.C.). Sweating was induced in 2 groups of young human male subjects in a heat cabinet for 6 hours daily on 5 consecutive days. One group was salt depleted prior to and during the study by means of a low salt diet; the other group was salt-loaded with 40 gm. of NaCl p.o. daily. In both groups NaCl excretion in the sweat reached maximum levels on the second day then fell on the third day and remained low on the fourth and fifth days. Although the concentration of NaCl in the sweat was considerably greater on the first and second days in the high salt group, the pattern of excretion of sweat NaCl in both groups was remarkably similar on the third, fourth and fifth days.

Skin biopsies taken before and after each day of sweating revealed similar morphologic changes in the cells of the eccrine sweat gland in both groups. After one day of sweating there was loss of glycogen from both the secretory coil and duct, vacuolization of the secretory cells, excretion of Schiff-positive, diastase-resistant material from the secretory cells and cellular atrophy. Before sweating on the second day, glycogen had reappeared in the secretory cells and no further depletion of this glycogen on subsequent days of sweating occurred. After sweating on the second day further vacuolization of the large pale cells in the secretory coil was observed. Many of the large pale cells contained two nuclei. On the third day glycogen reappeared in the cells of the duct and Schiff-positive, diastase-resistant material was observed in the secretory cells. After 6 hours of sweating further vacuolization and atrophy of the large pale cells occurred. By the fourth day a definite decrease in the population of large pale cells was apparent. Many of the pale cells had 6 to 8 nuclei, many of which were pyknotic. After sweating on the fifth day some secretory coils showed considerable recovery toward normal. Others revealed further distortion, atrophy, vacuolization and nuclear changes.

It is postulated that acclimatization may be dependent on the cytologic changes which occur in the sweat gland as a result of repeated profuse sweating and may be independent of increased adrenal cortical activity, since it occurs in the absence of salt depletion.

Activity of Sweat Glands Under the Influence of Various Environmental Stimuli. STEVEN M. HORVATH, PH.D. (Division of Research, Lankenau Hospital). The human organism attempts to remain in a state of heat balance, not a state of equilibrium but a *semi quasi* sort of steady state. Both sides of the equation of the steady state, heat production and heat loss, are variable quantities, depending on both physiological and physical parameters.

The heat production varies much more with physiological than with physical environmental factors. The heat production can only increase three-to fourfold for long continuous periods and can periodically be elevated eight- to twelvefold for very short periods of time. In contrast the heat loss depends more upon the physical factors of the environment as do, in some instances, increases in body heat content.

The functional capacity of the sweat glands under a variety of environmental stresses, from extreme cold to extreme heat and humidity, was discussed. Variations in volume and composition of the sweat produced was related to the duration of exposure and the type of environmental stress. Some of the questions relating to the so-called "fatigue" of sweat glands were considered.

It has not been my intention to develop all of the many ideas and contentions regarding the manner in which the sweat glands respond to thermal stresses. The physiology of these structures is still unknown and much more has to be learned before it is possible to evaluate their mechanism of action and the factors which modify their responses. This may well be accomplished in the near future if the interest of investigators in this field remains at its present level.

Physiology of Apocrine Sweat Gland. HARRY J. HURLEY, M.D. (Department of Dermatology, Hahnemann Medical College, Philadelphia, Pennsylvania). In man, apocrine sweat glands represent atavistic cutaneous appendages. These glands are generally restricted in man to the axillae, ano-genital skin, mammary areola and ear canal (wax glands). Almost always associated with hairs, apocrine sweat glands are roughly ten times larger than the eccrine sweat glands and show considerable racial variation, anatomically and physiologically. Histologically, apocrine tubules possess a single type of secretory cell with a well-developed myoepithelium investing the secretory coil. Apocrine sweat glands receive a single, adrenergic innervation.

Although present at birth, apocrine sweat glands do not mature and begin to function until puberty. They tend to involute following the climateric and show variable and inconsistent alterations in morphology and activity during various endocrine states, such as pregnancy.

Apocrine sweat gland function, like that of the mammary gland, can be divided into two distinct phases: (1) apocrine secretion, the formation of apocrine sweat in the tubules below, and (2) apocrine sweating, the appearance of apocrine sweat on the skin surface. Apocrine secretion is a slow, more or less continuous process, which is apparently under endocrinologic control. Although a pituitary principle is suspected, the precise hormone or hormones governing apocrine secretion have not yet been identified. Once formed, apocrine sweat resides in the tubules until the myoepithelial cells are stimulated to contract expressing the sweat to the skin surface. Pharmacologically, myoepithelial contraction (and thus apocrine sweating) may be induced by adrenergic drugs (epinephrine, norepinephrine) and pitocin, the oxytocic principle of the posterior pituitary which similarly stimulates mammary gland myoepithelium to result in "let-down" of milk. Apocrine sweating may be blocked by adrenergic blocking agents, but is unaffected by cholinergic and anticholinergic drugs. Apocrine sweating is *not* seen in response to *heat*, but usually appears after moderate to intense emotional (fear, anger) stimuli and painful sensory stimuli.

Apocrine sweat is generally turbid-white in appearance although it may occasionally be colored. Yellow, blue or green represent the usual color variants. Significantly, apocrine sweat, which contains protein, lipids, carbohydrates, ammonia and iron, is odorless and sterile when it initially appears on the skin surface. It is only after bacterial decomposition that the characteristic acrid odor of apocrine sweat develops.

BOOK REVIEWS AND NOTICES

THERAPEUTIC RADIOLOGY: RATIONALE, TECHNIQUE, RESULTS. BY WILLIAM T. MOSS, M.D., Assistant Professor of Radiology, Northwestern University School of Medicine. Pp. 403; 146 ills. St. Louis: C. V. Mosby Co., 1959. Price, $12.50.

AMERICAN Radiotherapy is doubly fortunate to have two superb books on therapeutic radiology make their appearance in 1959. The present textbook by Moss shows a need not previously supplied by any textbook in therapeutic radiology published either here or abroad. The particular vacancy so admirably filled in this case is that of presenting results accomplished by radiation therapy in the hands of great radiation therapists over the world, as an integral part of discussion of the principles and practice of radiation therapy of each of the specific lesions, chapter by chapter. In this respect, alone, the book is an invaluable asset to every department dealing with the care of tumors by any modality, since it is possible to see by reference to the tables of results, what can be accomplished by radiation therapy.

The book is further distinguished by excellent sections in each organ system, and in each tumor site, devoted to the philosophic considerations, guides to treatment dosage, the radiobiologic aspects to such treatment, and the clinical responses not only of the tumor, but of the adjacent normal tissues, to the radiation dosage required for the treatment of the tumor. It is a tribute to the pureness of the author's style that he has been able to accomplish all of this within the confines of a relatively small book, already an overwhelming favorite of all students of therapeutic radiology. R.R.

RADIATION THERAPY. BY WALTER T. MURPHY, M.D., Director of Therapeutic Radiology, Roswell Park Memorial Institute, Buffalo. Pp. 1041; illustrated. Philadelphia: W. B. Saunders Co., 1959. Price, $25.00.

AFTER nearly a decade during which American Radiotherapy has not been represented by a textbook, we are in a fortunate position of having two superb contributions in this field, the latest which is that by Murphy, Director of Radiotherapy, Roswell Park Memorial Institute, Buffalo, New York. This book is encyclopedic in scope, covering in admirable detail not only the physical and the philosophical aspects of radiation therapy of cancer, but also the specific aspects of therapy of individual lesions. In this respect it is notably different from all of the other radiotherapy books known to me, including those of British origin, because of the great detail with which individual specific problems of tumor therapy for all sites are discussed. For while other radiotherapy books are content to discuss broad principles of therapy by site and region and tumor (also adequately covered by Murphy), Murphy takes individual specific problems, and typical radiotherapy situations, and presents in great detail the method by which he has treated these lesions. As such, the textbook is a gold mine of information and support to the radiologist who finds himself in need of counsel and advice on specific problems rather than generalities. The book thus establishes itself as a requirement for everyone dealing with the treatment of malignant disease, by any modality. R.R.

AN EXPERIMENTAL INQUIRY INTO THE PRINCIPLES OF NUTRITION AND THE DIGESTIVE PROCESS. BY JOHN R. YOUNG, M.D., with an introductory essay by WILLIAM C. ROSE. Pp. 48. Urbana, Ill.: University of Illinois Press, 1959. Price, $2.50.

AFTER spending 8 months at the University of Pennsylvania School of Medicine, John R. Young, in 1803, wrote a remarkable thesis on his own observations and investigations of nutrition and digestion.

The first part of the essay is devoted to a philosophical consideration of nutriments. Most of the statements reflect concepts accepted at the time but the author does not hesitate to take issue with authorities if he finds their thinking illogical. The second part of the thesis is devoted to the authors investigations of gastric digestion. He selected the large bullfrog as the major subject of his experiments because of the spaciousness of its stomach and the accessibility of its gastric contents. Methodically he tested the gastric digestion of smaller animals, meat, vegetables and the like, until he exploded the fermentation theory of gastric digestion. He attempted to identify chemically the gastric acid and, with clever experimentation, came to a wrong conclusion. The design of experiments to demonstrate the lack of digestion of viable

tissue showed great imagination. Experiments were conducted also on other animals and on humans. It took courage to oppose the current thinking of his day but the author states, "Experiments are the sure and unerring guides to truth, when they alight the path we ought with confidence to pursue it, though authority should oppose."

For over 100 years this historically important manuscript went unnoticed but recently has been reproduced under the sponsorship of the University of Illinois History of Science Society. Supplementing this thesis is a delightful introduction by William C. Rose who provides interesting data on the personal background of our young investigator as well as pertinent information regarding his teachers, peers and the medical knowledge of his day. Rose points out this thesis was written 22 years before the discovery of hydrochloric acid in the gastric fluid and 30 years before Beaumont's monograph. Our gratitude is extended to all who made the republishing of this essay possible so that those fortunate enough to read it may be made familiar with the experimental work of a man who probably should be regarded as the Father of American gastrointesinal physiology. S.L.

THE CLINICAL EVALUATION OF NEW DRUGS. Edited by S. O. WAIFE, Indiana University School of Medicine, and A. P. SHAPIRO, University of Pittsburgh School of Medicine. Pp. 223; 1 ill. New York, Paul B. Hoeber, Inc., 1959. Price, $7.50.

THIS book was prepared by 14 authors, experienced as both teachers and researchers, and representing both industry and medical schools. The book is a timely one concerned with a rapidly developing field of great importance to progressive students and practitioners as well as teachers and researchers. The book deals particularly with the experimental methods necessary and appropriate for the proper clinical evaluation of new drugs. Part I of the book deals with general principles and includes discussion of such problems as experimental design, statistical analysis, use of human beings as experimental subjects, human volunteers, placebos, ethics and the training, responsibility, and reliability of the investigator. Part II deals more specifically with the problems of clinical drug evaluation in specific areas of practice: infectious disease, cardiovascular disorders, gastrointestinal diseases, nutrition and metabolism, endocrinologic problems, and neuropsychiatric disorders. Anyone attempting to do clinical investigation or to draw conclusions from clinical research reports would do well to read this book. It is not an authoritative answer to all problems in this complicated field, nor does it express a unanimous opinion of all qualified clinical investigators who have contributed to the advance of human health and knowledge. It does express the views of a number of well qualified investigators. R.S.

MEDICINAL CHEMISTRY, VOLUME IV. Edited by F. F. BLICKE AND R. H. COX. Prepared under the auspices of the Division of Medicinal Chemistry, American Chemical Society. Pp. 334. New York: John Wiley & Sons, Inc., 1959. Price, $12.00.

THE fourth volume in this series is entitled "Barbituric Acid Hypnotics," and is authored by William J. Doran, Eli Lilly and Company, Indianapolis. The text itself occupies only the first 42 pages, including tables related directly to the text; Addenda, pages 44-47, list of barbituric acids alphabetically by generic name and by trade name. Pages 48-273 contain a tabular classification of barbiturates and of the tables, of which there are 230. The book is documented by an extensive list of references. A large formula index is provided in addition to a subject index. The text is brief and to the point, summarizing the chemistry and pharmacology of barbituric acid and its derivatives. This volume, as for other volumes in the series, should prove to be of particular value to research chemists and pharmacologists concerned with the development and evaluation of new drugs. R.S.

RESPIRATORY PHYSIOLOGY AND ITS CLINICAL APPLICATION. BY JOHN H. KNOWLES, M.D., Massachusetts General Hospital and Harvard Medical School. Pp. 256; 8 ills. Cambridge, Mass.: Harvard University Press, 1959. Price, $5.25.

TESTS of respiratory function require cumbersome apparatus and complicated mathematical formulae. Give a physiologist a blackboard or the opportunity to show slides and before long the usual medical audience is either bewildered or asleep. That mythical creature, "the average medical reader" is apt to be similarly confused by much of the current literature concerning respiratory physiology. Dr. Knowles has attempted, on the whole very successfully, to present the large and important current body of information in an understandable fashion. The first section

of this well written book describes normal respiratory physiology and the available battery of pulmonary function tests. The indications, limitations, normal variations, and potential errors of the various procedures are discussed. Whenever possible the simpler tests are stressed, since they are more readily available and easier to understand. Part II discusses the pathological physiology of the important diseases and conditions that affect respiration. Emphysema, asthma, air cysts, as well as extra-pulmonary factors such as pregnancy, neuromuscular disorders, heart disease and aging receive appropriate attention. Differential diagnosis is clarified by excellent tables. As in Part I the simpler tests are recommended whenever they can furnish the necessary information concerning diagnosis, prognosis, or response to treatment.

An excellent bibliography, 426 references, and index add to the value of this splendid volume, which more than fulfills the author's description of "a primer for the interested neophyte." R.C.

PHARMAKOLOGIE: ALS THEORETISCHE GRUNDLAGE EINER RATIONELLEN PHARMACOTHERAPIE. BY KNUD O. MÖLLER, Professor of Pharmacology, University of Copenhagen. 3rd ed., expanded. Translated and edited from the fifth Danish edition by DR. O. WALKER, Basel. Pp. 902; 50 ills. Basel, Switzerland: Benno Schwabe & Co., 1958. (Distributed in U.S.A. and Canada by Intercontinental Medical Book Corporation, New York.) Price, $16.25.

THIS expanded and revised third German edition, intended as a basis for rational Therapeutics, is similar in intention, scope, and general arrangement of subject matter to the larger American textbooks. For those who read German it should prove suitable as a textbook of pharmacology and as a basic reference book. The third edition has been expanded to include the many new developments in pharmacology since the second edition in 1952, including psychomimetic and psychotherapeutic drugs, oral hypoglycemic agents, carbonic anhydrase inhibitors, serotonin, the newer antibiotics, corticosteroid derivatives, and numerous others. Pertinent references appear at the end of many of the individual sections within chapters and there is, in addition, an extensive reference list occupying 57 pages at the end of the book. The book is also well indexed. A brief but excellent section on chemical formulae and terminology is presented in the chapter on general principles. Careful attention is paid to basic definitions and concepts. Coverage is brief in many respects, but probably adequate, and a relatively good balance between the old and the new is achieved. R.S.

CLINICAL AUSCULTATION OF THE HEART. BY SAMUEL A. LEVINE, M.D., Sc.D.(HON.), Peter Bent Brigham Hospital, and W. PROCTOR HARVEY, M.D., Georgetown University Hospital. 2nd ed. Pp. 657; 660 ills. Philadelphia and London: W. B. Saunders Company, 1959. Price, $11.00.

Two clinicians, masters of the art of auscultation, intensively describe in their own inimitable manner the clinical characteristics of cardiac sounds and murmurs. The advent of modern technology has not minimized clinical stethoscopy. On the contrary, stethoscopy makes meaningful an abstract technology that in turn becomes dynamic when viewed in the light of clinical findings. The authors use phonocardiography only to illustrate specific points and not as a method of investigation *per se*. This is unfortunate because phonocardiography can contribute much to the clinical detection and understanding of cardiac sounds and murmurs, as has been so elegantly demonstrated by Leatham, and to the analysis of sound composition as shown by McKusick, and to the potential functional significance of cardiac sounds as studied in relation to other parameters by Wells. Nevertheless, with knowledge of their findings and with persistent practice, the stethoscope can be made to work for us. To paraphrase Auenbrugger, one cannot hear until one has learned to hear, and in this book one can find what to hear, how to hear it and make that which is heard clinically meaningful. J.Z.

SECHZIG JAHRE MEDIZINISCHER RADIOLOGIE. PROBLEME UND EMPIRIE. BY HANS R. SCHINZ, Zurich. Pp. 275; 86 ills. Stuttgart: Georg Thieme Verlag, 1959 (Intercontinental Medical Book Corp., New York, U.S. Agents). Price, $4.65.

THIS little book, excellently arranged, was presented to each member of the International Congress of Radiology by the Georg Thieme Verlag. Schinz may be one of the very few left, who participated in the historical development of radiology through his scientific work and personal friendships with leading pioneers of the science of radiology. This personal experience makes his account especially enjoyable. The book describes concisely and critically the various research eras,

namely, physical invention (with reproduction of the original papers of Roentgen, Becquerel, Curie, Rutherford, Hahn and Strassman), biology of rays, diagnostic roentgenology, radio and electronic therapy, and others, with short curriculum vitae and picture of the pioneer in question. Thus, the book represents a mine of historical data for each radiologist, medical historian, or well educated person. One can only be grateful to Dr. Hauff (owner of the Georg Thieme Verlag), for this generous present. W.B.

Die Prophylaxe der Schlafstoerung. By Prof. Dr. Paul Vogler. 2nd ed. Pp. 130. Leipzig: Georg Thieme Verlag, 1959. Price, DM 15.00.

The second edition of "Prophylaxis of Sleep Disturbances" was revised and brought up-to-date in cooperation with Wolfgang Pfeiffer of Bandung, Java. It is a timely and practical contribution to the vital topic of insomnia. The manifold somatic and environmental causes of this condition are discussed on the basis of well selected case histories.

The therapeutic approach, specifically the psycho- and hydrotherapeutic measures, is dealt with in great detail in separate chapters. The frequently neglected factors contributing to sleeplessness, such as inadequate nutrition, poor ventilation, general climate and the microclimate of the bedroom are presented in a clear and concise manner.

An extensive bibliography enhances the value of this interesting monograph. General practitioners, psychiatrists and physiatrists will find here much useful information.

I.K.

Strahlenbiologie. Grundlagen und Ergebnisse. By Prof. Dr. H. Fritz-Niggli, Zurich. Pp. 379; 168 ills. Stuttgart: Georg Thieme Verlag, 1959 (Intercontinental Medical Book Corp., New York, U.S. Agents). Price, $15.50.

This book is an unique and fundamental aid for all who are interested in radiobiology, genetics, pathology and death as it concerns radiation. Extremely comprehensive knowledge and numerous research data have been compiled with stupendous diligence, and then have been critically analyzed. This book will be especially appreciated at this time, when genetic damage is being recognized and laws are being instituted for protection from radiation. A comprehensive 30-page bibliography is included. The quality of the publication is excellent. W.B.

Les Méthodes en Génétique Générale et en Génétique Humaine. By Roger Huron and Jacques Ruffié. Pp. 556; 79 ills. Paris: Masson et Cie, 1959. Price, 8.200 fr.

This textbook of genetics, printed in French, presents lucid explanations, with well constructed illustrative diagramatic drawings and with both theoretical and actual illustrative examples of the basic principles of genetics. The book is divided into a section on general genetics, including a brief but pointed statement of the concept of cytoplasmic heredity (plasmagenes) and geneplasmagene-virus interrelationships; a section on heredity in man, in which biologic methods, mathematical methods, the binomial law and its limitations, and correction factors are considered; a section on an introduction to population genetics; and a section on specific problems of human genetics which includes a discussion of genetic counseling.

The reviewer considers this book an excellent, straightforward presentation of a subject of ever-increasing importance in medical science. H.W.

Acta de L'Institut D'Anesthesiologie. By P. Moulonguet and J. Baumann de L'Institut D'Anesthesiologie. Vol. 7, 1958. Pp. 260; 27 ills. Paris: L'Imprimerie Clerc Saint Amand, for the Library Arnette, 1958. Price, 2.500 fr.

This is an extremely well written book in which P. Moulonguet and J. Baumann with the collaboration of twelve other authors present material of special interest to all anesthesiologists. The chapters are well documented with tables, charts, case histories, and bibliographies.

There are several chapters of great interest. The first chapter discusses preoperative anxiety and the significance of proper preoperative evaluation including psychotherapy where indicated.

There is a section devoted to the use of the electroencephalogram and its interpretation during anesthesia. Many tracings of EEG patterns are shown as associated with various physiopathologic states such as carotid occlusion, hypotension and hypothermia.

Two chapters, which are very well written, present material concerning the effects of various pathologic conditions upon pulmonary ventilation. These topics include: massive thoracic trauma, poliomyelitis, tetanus, postoperative hypoventilation, and electrolyte imbalance.

Other sections which should be mentioned are those dealing with the management of

circulatory collapse, the indications for using the artificial kidney, the problems encountered when anesthetising patients for surgery about the mouth and face, anesthetic explosions, and postoperative neurologic complications. L.K.

ENDOCRINOLOGY OF REPRODUCTION. Proceedings of the Conference held in Syracuse, New York, June 9-12, 1958. Edited by CHARLES W. LLOYD. Pp. 532; illustrated. New York & London: Academic Press, Inc., 1959. Price, $12.00.

RECENT progress in the Endocrinology of Reproduction is a compilation of the papers presented at a conference sponsored by the Upstate Medical Center, State University of New York. They were edited and prepared for publication by Charles W. Lloyd of that institution. The participants, each of whom is a recognized authority in his field, include biologists, chemists, anatomists, physiologists, endocrinologists and veterinarians, as well as clinicians. The material presented and discussed was diversified but unquestionably related to some phase of reproduction. The subject matter includes the mechanism and control of ovulation, estrogen metabolism and excretion, the production and transport of sperms, progestational activity, placental function, action of the neurohypophysis, relaxia and mammary growth and lactation.

Although many of the papers concern basic research there is much information which can be applied to clinical practice; this is particularly true of the material concerning sperm production, estrogen metabolism and the gestagens. This volume should therefore be of value both to investigators in the field of reproduction and to practicing obstetricians and gynecologists. J.W.

THE DIABETIC'S HANDBOOK. BY ANTHONY M. SINDONI, JR., M.D., Chief, Department of Metabolism, St. Joseph and Philadelphia General Hospitals. 2nd ed. Pp. 285; 9 ills. New York: The Ronald Press Company, 1959. Price, $4.50.

THE diabetic patient has been able for many years to obtain manuals prepared by experts in this field to improve the level of self-care of his disease. This vital adjunct in the management of a specific illness is being extended gradually to other medical sub-specialities. This volume provides an adequate fund of general information concerning the practical aspects of the treatment of diabetes. A number of collaborators have contributed brief commentaries upon certain aspects of diabetes and its management; one of these dealing with the emotional aspects of diabetes by Poinsard is outstanding. While this manual covers the topic admirably, the selection of a text for patients should be made by each physician to provide details coinciding with his programs of treatment. C.S.

THE SURGEON AND THE CHILD. BY WILLIS J. POTTS, M.D., Surgeon-in-Chief, Children's Memorial Hospital; Professor of Pediatric Surgery, Northwestern University Medical School, Chicago. Pp. 250; illustrated. Philadelphia: W. B. Saunders Co., 1959. Price, $7.50.

THIS is a nice little book that brings out the high points of some of the surgical situations that are met in children. The material is covered concisely and is easy reading. Dr. Potts succeeds in including a part of himself in this book which in turn makes the book all the more valuable.

THIS is not by any manner of means a complete text on pediatric surgery but most of the essentials are covered. R.T.

DAS ROENTGENSCHICHTBILD. BY A. GEBAUER, E. MUNTEAN, E. STUTZ AND H. VIETEN. Pp. 446; 699 ills. Stuttgart: Georg Thieme Verlag, 1959 (Intercontinental Medical Book Corporation, New York, U.S. Agents). Price, $35.25.

THE first part covers completely the basic geometrics of tomography including technique. The second part delineates the range of indication for this technique for all organs, starting with the skull. The chapters on the chest are very impressive. Then follow valuable data about the central nervous system, localization of foreign bodies, and finally, tomography as an aid in roentgentherapy. All chapters have been written by outstanding specialists. The abundant bibliography is international. Quality of paper, illustrations, printing and binding prove the high standard of a product of the Verlag Georg Thieme. W.B.

LES STRUCTURES INFRAMICROSCOPIQUES NORMALES ET PATHOLOGIQUES DES CELLULES ET DES TISSUS. BY A. POLICARD AND C. A. BAUD. Pp. 476; 138 ills. Paris: Masson et Cie, 1959. Price, 5.200 fr.

THIS volume is a clear and concise presentation of ultrastructure of cells and tissues

with emphasis on the normal, and including mention of pathologic alterations. The volume, therefore, is a start at filling a serious need in the very rapidly expanding fields related to ultrastructure research, and as such, it is considered a most valuable work. While treatment of many subjects is necessarily brief, a fine survey of present (up to 1957-1958) knowledge and studies in ultrastructure is presented. Most of the illustrations are line drawings, supplemented by photographs, generally of excellent quality. The use of drawings is in itself a helpful method in presenting fine structure especially to the reader not already familiar with interpretation of electron micrographs. Some sections would be enhanced by more illustrations.

The book is divided into three main sections: Methods, including interpretation of electron micrographs, a discussion of artefacts, staining methods and auxiliary methods (Roentgen-ray diffraction, polarized light); the cell, with discussions of ultrastructure generally applicable to all cells; and tissues, a systemic review which includes most of the organ systems.

The text is in French. It is a fine book and should serve the microscopist as a good starting point in considering cell ultrastructure, generally or in a particular organ or tissue.

H.W.

CLINICAL COORDINATION OF ANATOMY AND PHYSIOLOGY. By MARTHA PITEL, PH.D., R.N.; University of Rochester (formerly of Yale University) AND MILDRED SCHELLIG, M.D.; State University of New York, Harpur College. Pp. 320; 101 ills. New York: Springer Publishing Company, Inc., 1959.

THIS combination workbook and laboratory manual is designed to answer the present day demand for "patient oriented" teaching whereby the student nurse sees the application of anatomy and physiology to her clinical experiences. Laboratory exercises are correlated with 57 case histories by questions asked following each section. This is an intriguing method wherein correlation can come to a student working with this manual, textbooks, ample time and a great deal of understanding guidance. Illustrations are simple, direct, functional. Although some minor errors occur (adductor for abductor, etc.) this is a substantial book for nursing education. R.H.

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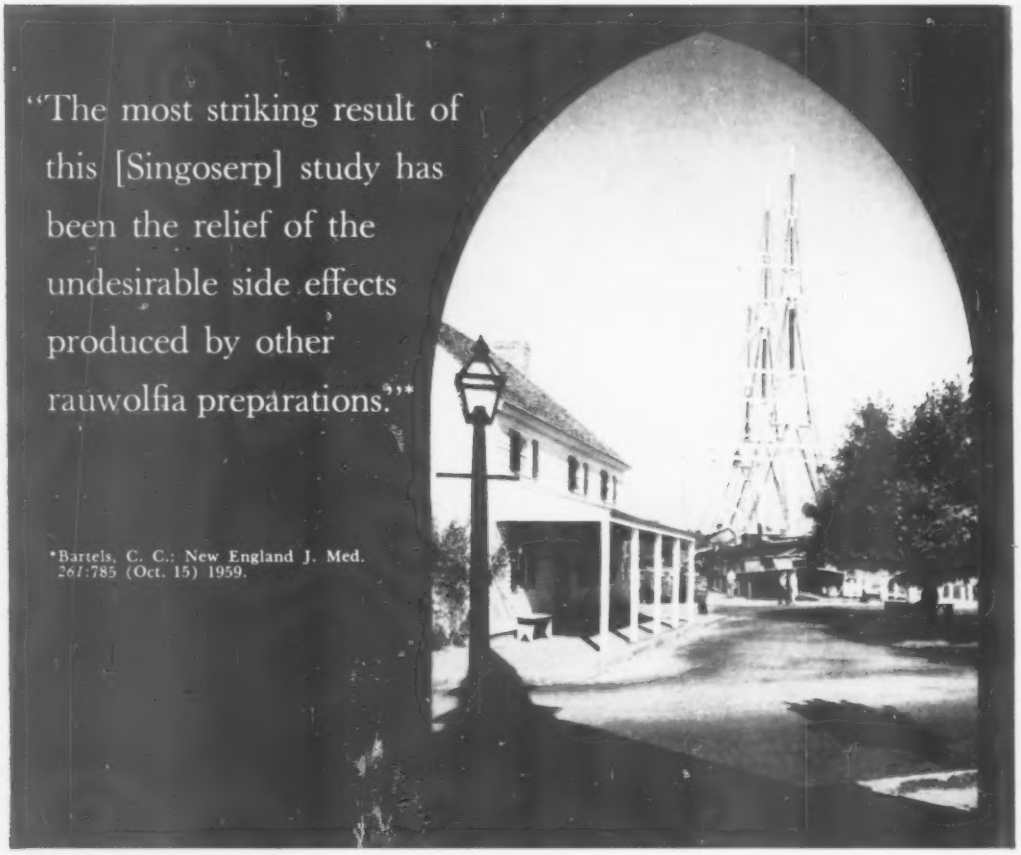
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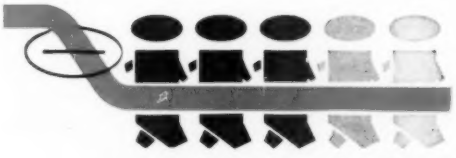

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F. K. Albrecht, Mod. Mgmnt. Clin. Med. P674, Williams & Wilkins.
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"Slow clearers" gradually accumulate an excess of fat in the blood stream over a period of years as each meal adds an additional burden to an already fat-laden serum. As shown in *figure #2,* the blood literally becomes saturated with large fat particles, presenting a dual hazard to the atherosclerotic patient: the long-term danger of deposition of these fats on the vessel walls,[4] and the more immediate risk of high blood fat levels after a particularly heavy meal possibly precipitating acute coronary embarrassment.[5]

In *figure #3,* the test tube at the left contains lipemic serum, while the one at the right contains clear, or normal serum. If serum examined after a 12-hour fasting period presents a milky appearance, this is a strong indication that the patient clears fat slowly and is a candidate for antilipemic therapy in an effort to check a potentially serious situation.

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References: **1.** Anfinsen, C. B.: Symposium on Atherosclerosis, National Academy of Sciences, National Research Council Publication 338, 1955, p. 218. **2.** Berkowitz, D.; Likoff, W., and Spitzer, J. J.: Clin. Res. 7:225 (Apr.) 1959. **3.** Stutman, L. J., and George, M.: Clin. Res. 7:225 (Apr.) 1959. **4.** Wilkinson, C. F., Jr.: Annals of Int. Med. 45:674 (Oct.) 1956. **5.** Kuo, P. T., and Joyner, C. R., Jr.: J.A.M.A. *163*:727 (March 2) 1957. **6.** Fuller, H. L.: Angiology 9:311 (Oct.) 1958. **7.** Shaftel, H. E., and Selman, D.: Angiology *10*:131 (June) 1959. **8.** Fuller, H. L.: Circulation *20*:699 (Oct.) 1959.

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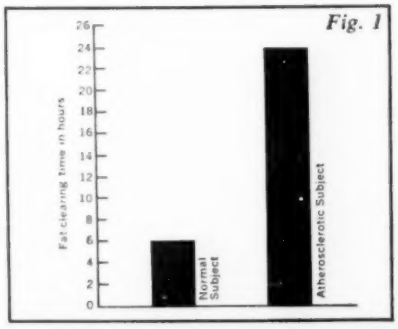

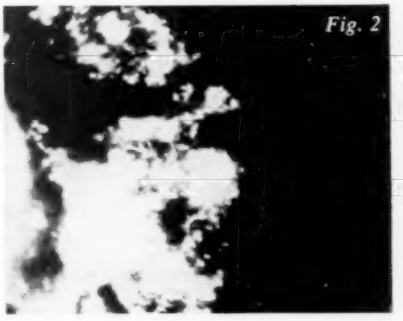

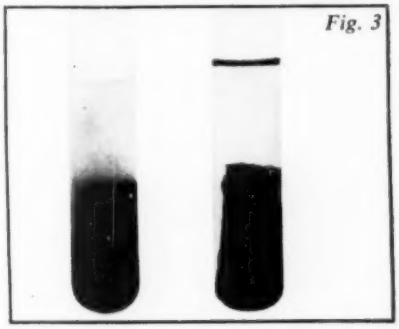

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1. Breneman, G. M., and Priest, E. McC.: Am. Heart J. 50:129 (July) 1955.

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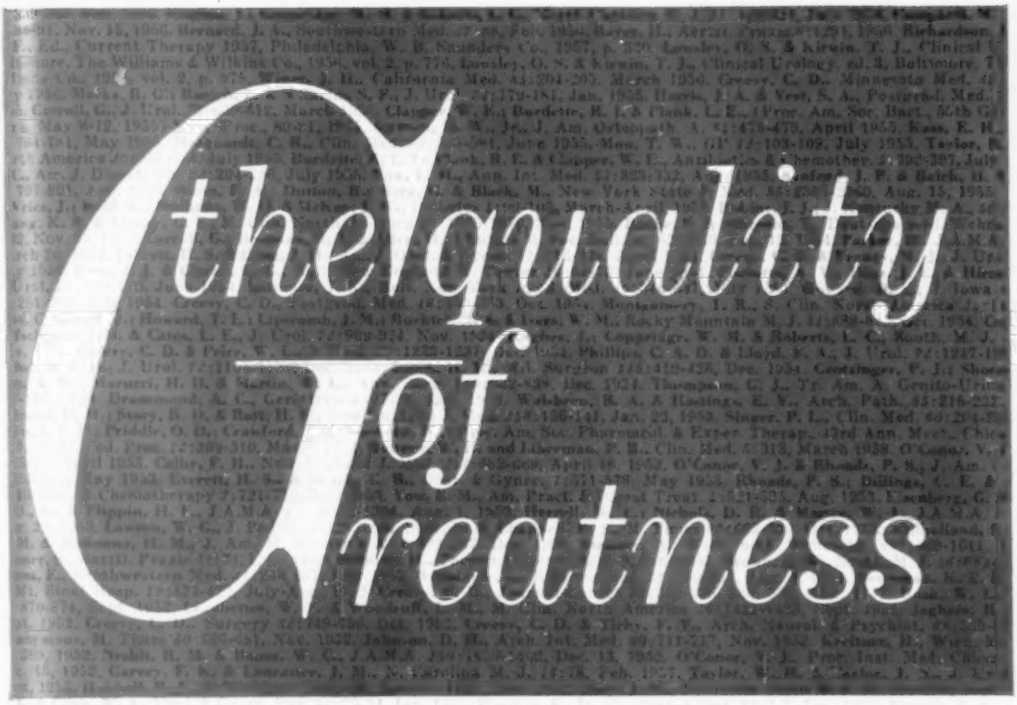

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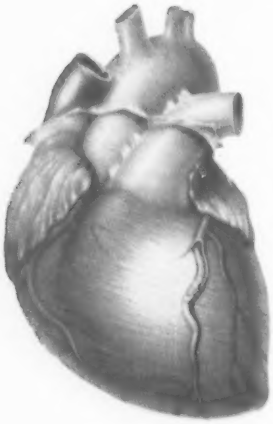

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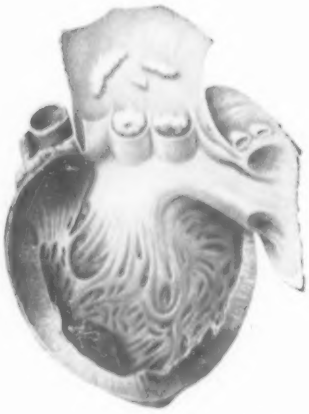

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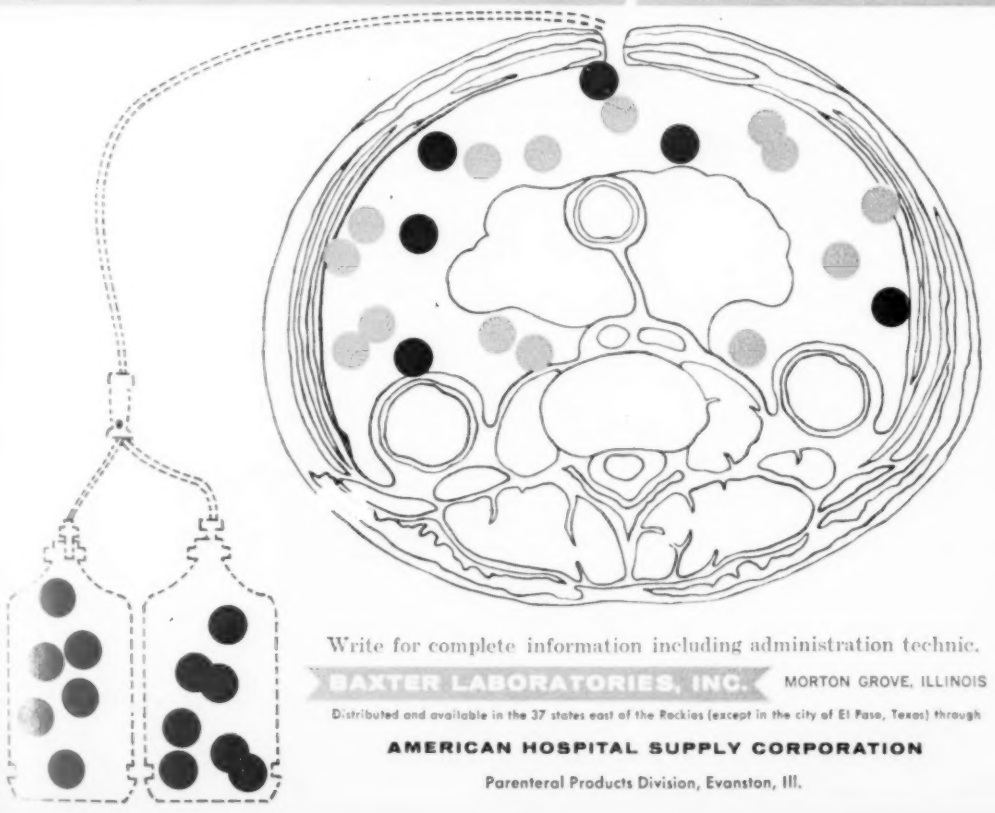

Write for complete information including administration technic.

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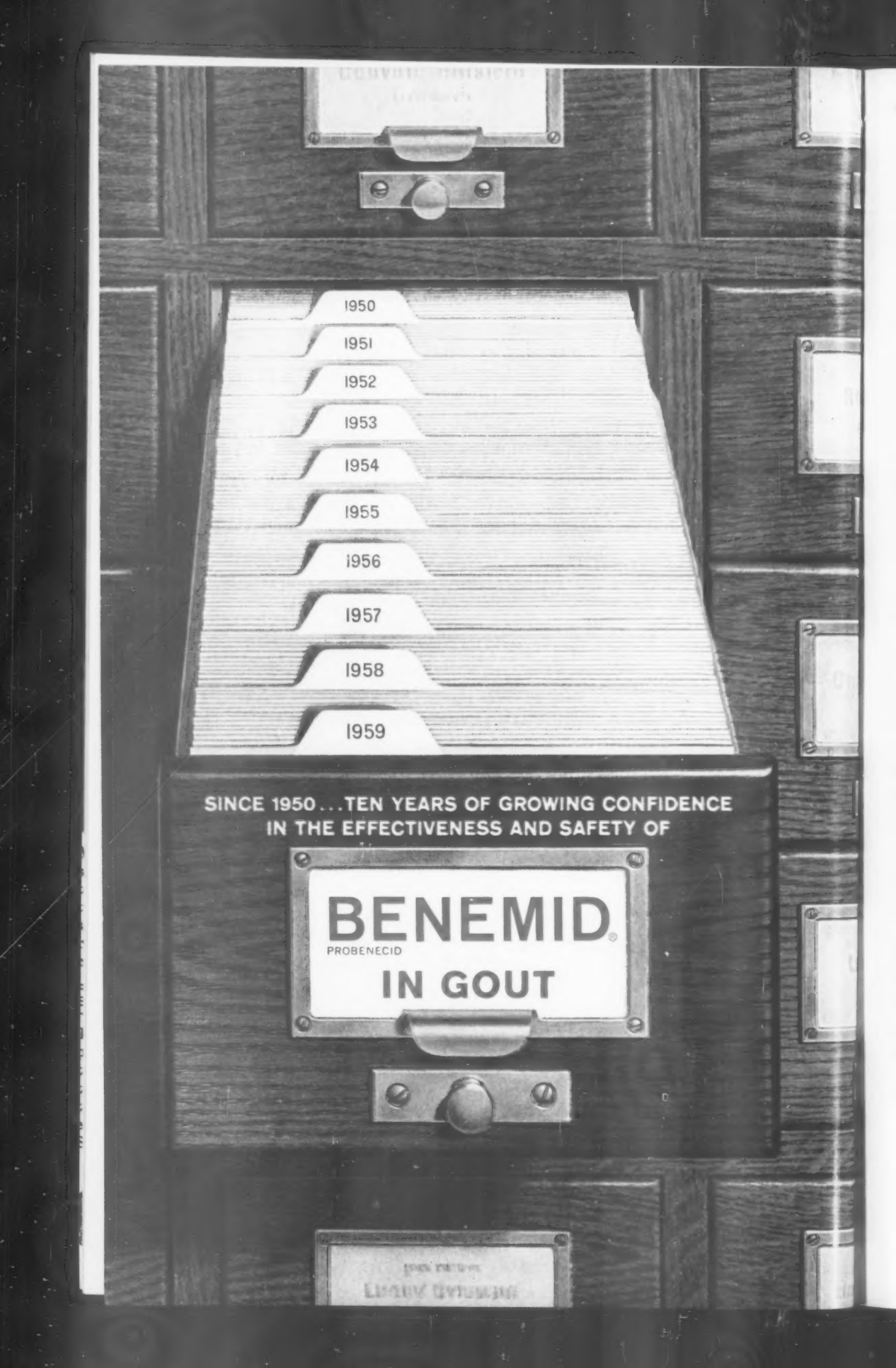
Distributed and available in the 37 states east of the Rockies (except in the city of El Paso, Texas) through

AMERICAN HOSPITAL SUPPLY CORPORATION

Parenteral Products Division, Evanston, Ill.


1950
1951
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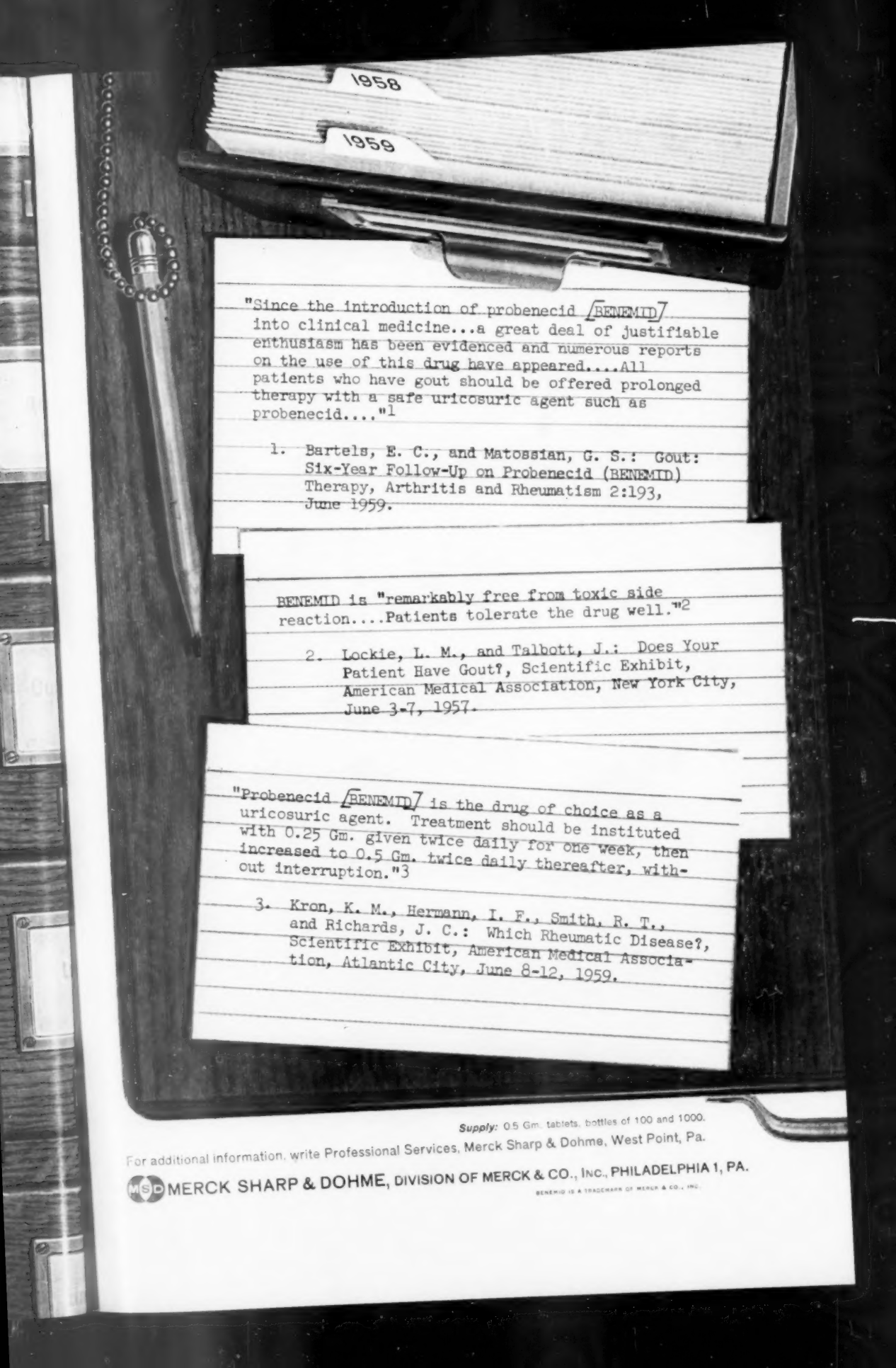
SINCE 1950 . . . TEN YEARS OF GROWING CONFIDENCE
IN THE EFFECTIVENESS AND SAFETY OF
BENEMID®
PROBENECID
IN GOUT



"Since the introduction of probenecid [BENEMID] into clinical medicine...a great deal of justifiable enthusiasm has been evidenced and numerous reports on the use of this drug have appeared....All patients who have gout should be offered prolonged therapy with a safe uricosuric agent such as probenecid...."[1]

1. Bartels, E. C., and Matossian, G. S.: Gout: Six-Year Follow-Up on Probenecid (BENEMID) Therapy, Arthritis and Rheumatism 2:193, June 1959.

BENEMID is "remarkably free from toxic side reaction....Patients tolerate the drug well."[2]

2. Lockie, L. M., and Talbott, J.: Does Your Patient Have Gout?, Scientific Exhibit, American Medical Association, New York City, June 3-7, 1957.

"Probenecid [BENEMID] is the drug of choice as a uricosuric agent. Treatment should be instituted with 0.25 Gm. given twice daily for one week, then increased to 0.5 Gm. twice daily thereafter, without interruption."[3]

3. Kron, K. M., Hermann, I. F., Smith, R. T., and Richards, J. C.: Which Rheumatic Disease?, Scientific Exhibit, American Medical Association, Atlantic City, June 8-12, 1959.

Supply: 0.5 Gm. tablets, bottles of 100 and 1000.

For additional information, write Professional Services, Merck Sharp & Dohme, West Point, Pa.

MSD MERCK SHARP & DOHME, DIVISION OF MERCK & CO., INC., PHILADELPHIA 1, PA.

BENEMID IS A TRADEMARK OF MERCK & CO., INC.

90% of anxious, agitated and apathetic office patients calmed without drowsiness and with normal drive restored...

on one or two 0.25 mg. tablets b.i.d.:

This is the pattern of performance for

PERMITIL®

Fluphenazine dihydrochloride

■ In 1164 patients with anxiety and anxiety-induced fatigue or depression, PERMITIL, administered in small daily doses of 0.5 mg. to 1 mg., produced significant improvement in 90%.*

■ PERMITIL is virtually free from side effects at recommended dosage levels.

■ Patients become calm without being drowsy and normal drive is restored.

■ Onset of action is rapid; effect is prolonged. ■ PERMITIL does not potentiate barbiturates or non-barbiturate sedatives and can be used with impunity with such agents.

How to Prescribe PERMITIL: The lowest dose of PERMITIL that will produce the desired clinical effect should be used. The recommended dose for most adults is one 0.25 mg. tablet twice a day (taken morning and afternoon). Increase to two 0.25 mg. tablets twice a day if required. Total daily dosage in excess of 1 mg. should be employed only in patients with relatively severe symptoms which are uncontrolled at lower dosage. In such patients, the total daily dose may be increased to a maximum of 2 mg., given in divided amounts. Complete information concerning the use of PERMITIL is available on request.

Supplied: Tablets, 0.25 mg., bottles of 50 and 500.

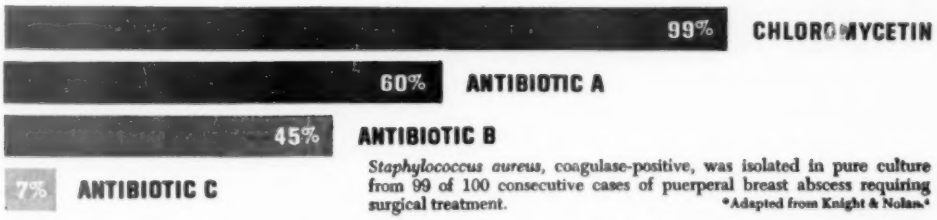
*Recent compilation of case reports received by the Medical Department, White Laboratories, Inc.

White WHITE LABORATORIES, INC., *Kenilworth, New Jersey*

repeatedly, clinicians report... effective results against staphylococci[1-4]

CHLOROMYCETIN®

IN VITRO SENSITIVITY OF STAPHYLOCOCCUS AUREUS TO CHLOROMYCETIN AND TO THREE OTHER ANTIBIOTICS*



Staphylococcus aureus, coagulase-positive, was isolated in pure culture from 99 of 100 consecutive cases of puerperal breast abscess requiring surgical treatment.

*Adapted from Knight & Nolan.[4]

CHLOROMYCETIN (chloramphenicol, Parke-Davis) is available in various forms, including Kapseals® of 250 mg., in bottles of 16 and 100.

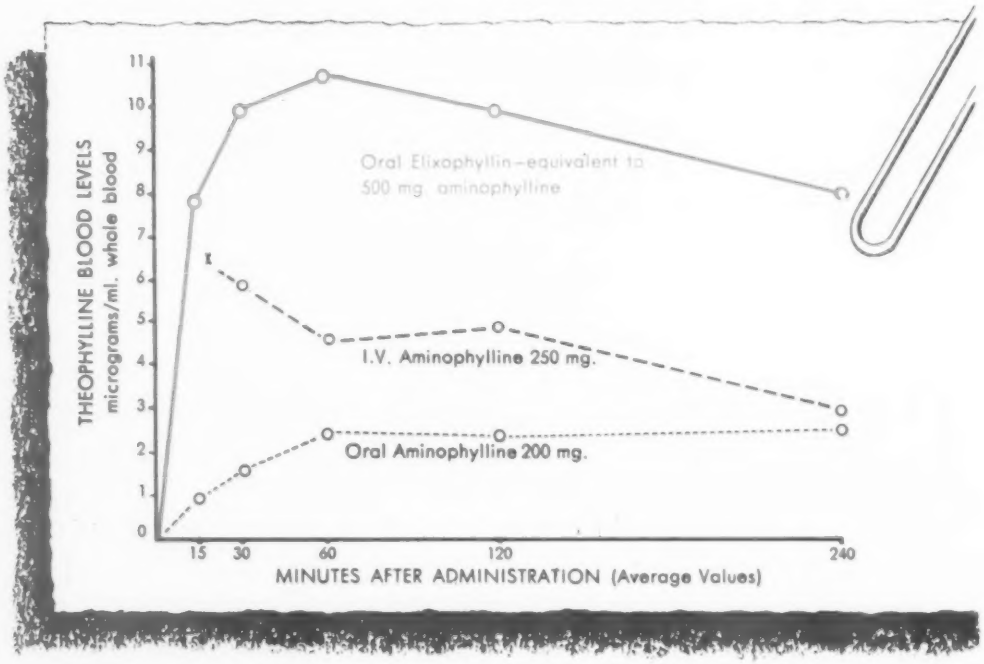
CHLOROMYCETIN is a potent therapeutic agent and, because certain blood dyscrasias have been associated with its administration, it should not be used indiscriminately or for minor infections. Furthermore, as with certain other drugs, adequate blood studies should be made when the patient requires prolonged or intermittent therapy.

References: (1) Finland, M.; Jones, W. F, Jr., & Bennett, I. L., Jr.: *Arch. Int. Med.* 104:365, 1959. (2) Welch, H., in Welch, H., & Finland M.: Antibiotic Therapy for Staphylococcal Diseases, New York, Medical Encyclopedia, Inc., 1959, pp. 14, 16. (3) Nichols, D. R., & Martin, W. J.: *Surg. Gynec. & Obst.* 107:523, 1958. (4) Knight, I. C. S., & Nolan, B.: *Brit. M. J.* 1:1224, 1959.

PARKE, DAVIS & COMPANY • DETROIT 32, MICHIGAN 33660 PARKE-DAVIS

asthmatic attacks terminated by a single oral dose

Because absorption is so rapid—speeded by the hydro-alcoholic vehicle—therapeutic theophylline blood levels are reached in minutes.



Adult dose for severe attacks is a wineglassful (75 cc. or 5 tbsp. containing theophylline equivalent to 500 mg. aminophylline). Well tolerated.

Elixophyllin data—Schluger, J., et al.: Am. J. M. Sci. 233:296, 1957; other data—Waxler, S. H. & Shack, J. A.: J.A.M.A. 143:736, 1950.

ELIXOPHYLLIN

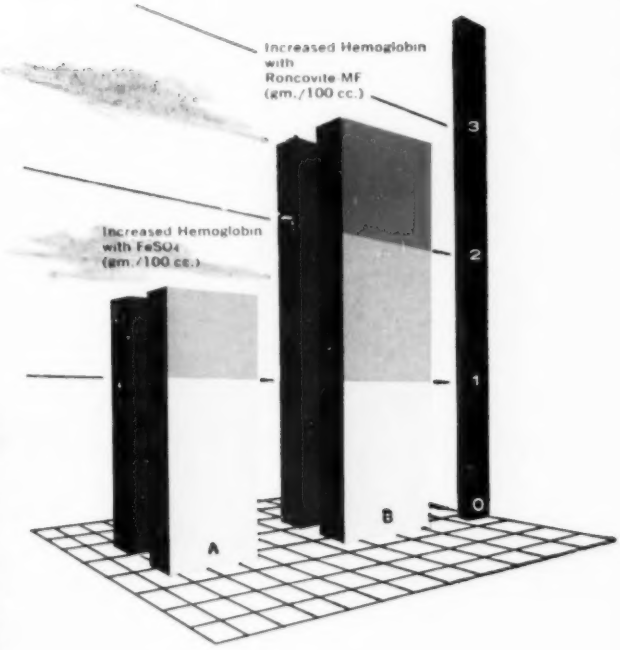
Sherman Laboratories

Literature on request *Detroit 11, Michigan*

LEGEND:

(A) Patients receiving ferrous sulfate 200 mg. q.i.d. showed average increase in hemoglobin of 1.5 gm.

(B) Patients receiving Roncovite-MF (15 mg. cobalt chloride and 100 mg. ferrous sulfate) showed average increase in hemoglobin of 2.7 gm.



Improved iron utilization in anemia

RONCOVITE®-mf

Each tablet contains: Cobalt chloride (Cobalt as Co....3.7 mg.)...15 mg. Ferrous sulfate, exsiccated...100 mg.

Improves iron utilization by enhancing the formation of erythropoietin, the erythropoietic hormone

Recent research[1,2] again emphasizes the role of cobalt as the only clinically proved agent which enhances erythropoietin formation.

The acceleration of erythropoiesis with Roncovite therapy provides optimal utilization of iron.

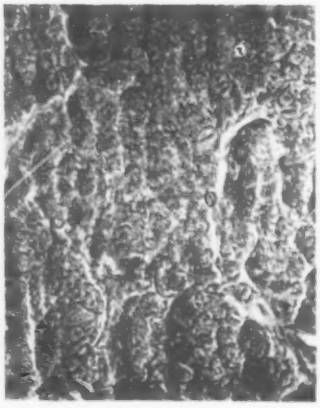
Acting through this physiologic mechanism, Roncovite (cobalt-iron) therapy results in an increased production of red cells and hemoglobin—a better blood picture—a faster, more complete response than iron alone in the common hypochromic anemias—menstrual anemia—anemia of pregnancy—nutritional anemia of infancy—and in anemia due to chronic infection or inflammation.[3,4,5,6,7,8]

(1) Goldwasser, E.; Jacobson, L. O.; Fried, W., and Plzak, L. F.: Blood *13*:55 (Jan.) 1958. (2) Gurney, C. W.; Jacobson, L. O., and Goldwasser, E.: Ann. Int. Med. *49*:363 (Aug.) 1958. (3) Korst, D. R.; Bishop, R. C., and Ethell, F. H.: J. Lab. & Clin. Med. *52*:364 (Sept.) 1958. (4) Ausman, D. C.: Journal-Lancet *76*:290 (Oct.) 1956. (5) Holly, R. G.: Obst. & Gynec. *9*:299 (Mar.) 1957. (6) Holly, R. G.: Clin. Obst. & Gynec. *1*:15 (Mar.) 1958. (7) Diamond, E. F.; Gonzales, F., and Pisani, A.: Illinois M. J. *113*:154 (April) 1958. (8) Hill, J. M.; La Jous, J., and Sebastian, F. J.: Texas J. Med. *51*:686 (Oct.) 1955.

LLOYD BROTHERS, INC. • CINCINNATI 3, OHIO

Prescribe enough Orinase* to release enough native insulin

Before:
Microphotograph showing insulin granules in beta cells of pancreas of normal dog.



During:
Degranulation following administration of Orinase. Note complete release of native insulin.

After:
Regeneration of granules following termination of Orinase dosage.

Adjust Orinase dosage to make available the amount of native insulin *needed* by the diabetic patient. This may be done freely because Orinase has virtually no "ceiling" imposed on dosage by toxicity or untoward effects.

In a series of 187 diabetic patients successfully managed on Orinase (tolbutamide) during a period of 6 to 30 months, the reported[1] distribution of daily doses was as follows: 1 gram, 17%; 1.5 grams, 22%; 2 grams, 40%; 3 grams, 21%.

Similarly, in three years' clinical experience with a population of approximately 3,000 diabetics on Orinase, it has been observed that about one-third of the patients at any one time require and receive dosages of 2 to 3 grams a day for successful management.[2]

To obtain optimum control, and avoid needless "secondary failures"—give sufficient Orinase to meet varying requirements from patient to patient or in a given patient from time to time.

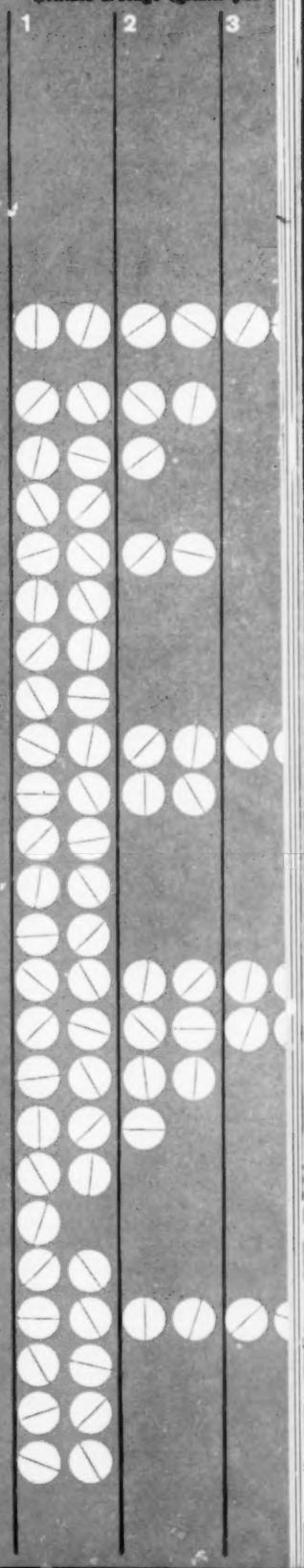
1. Gorman, C. K., and Weaver, J. A.: Brit. M. J. 2:1214 (Dec. 5) 1959.
2. Case data courtesy Henry Dolger, M.D.

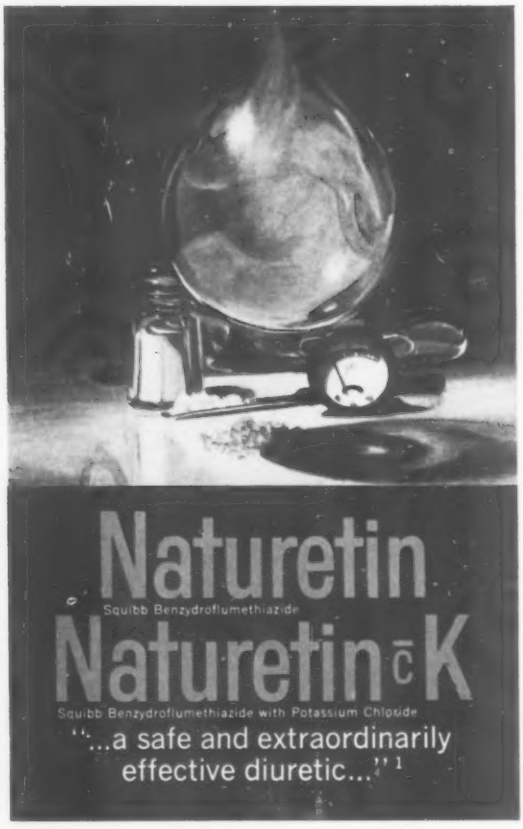
*TRADEMARK, REG. U. S. PAT. OFF.—TOLBUTAMIDE, UPJOHN

Upjohn THE UPJOHN COMPANY KALAMAZOO, MICHIGAN

a three-year case history[2]

Date	Entries and Comments
6/21/56	Mr. J. S., 54 yrs. old, diabetes mellitus 1¼ yrs. F. H.—neg. for diabetes. P. H.—surg. 0, med. 0. Restaurant manager—M., 3 children living and well. Wt. 155 lb. 1 yr. ago; now 125. P. I.—onset 1¼ yrs. ago with thirst and polyuria; glycosuria found. On diet. In past year lost 30 lb., strict diet—all kinds of dietetic substitutes. Some asthenia. Afraid of insulin. No recent glycosuria or nocturia. P. Exam.—thin male. Fundi neg. ENT neg. BP 140/80. Heart and lungs neg. Extremities: poor pulses. Urine—sugar 0, acetone 0. Noon blood sugar 240.
	Rx more adequate diet and Orinase 3 Gm.
7/9/56	Wt. 127, urine 0-0. B. S. 110, occ. nocturia but no glycosuria. Rx: eat more, Orinase 2 Gm.
8/29/56	Wt. 139½, urine 0-0-0. B. S. 205. Rx 1.5 Gm.
10/1/56	Wt. 143, urine 0-0-0. B. S. 125. Rx 1 Gm.
11/5/56	Wt. 148, urine 4+ -0-0, noon B. S. 160. Rx 2 Gm.
12/7/56	Wt. 146, urine 0-0-0, noon B. S. 120. Rx 1 Gm.
1/11/57	Wt. 144½, urine 0-0-0, noon B. S. 150. Rx 1 Gm.
3/11/57	Wt. 144½, urine 0-0, noon B. S. 120. Rx 1 Gm.
5/10/57	Wt. 140½, urine 4+ -0-0. B. S. 275. Rx 3 Gm.
6/12/57	Wt. 138½, urine 0-0-0, noon B. S. 114. Rx 2 Gm.
8/7/57	Wt. 136½, urine 0-0-0. B. S. 100. Rx 1 Gm.
10/2/57	Wt. 136½, urine 0-0-0. B. S. 85. Rx 1 Gm.
11/29/57	Wt. 136½, urine 0-0-0. B. S. 123. Rx 1 Gm.
1/17/58	Wt. 134½, urine 4+ -0-0. B. S. 216. Rx 3 Gm.
2/14/58	Wt. 132, urine 0-0-0. B. S. 135. Rx 3 Gm.
3/28/58	Wt. 136½, urine 0-0-0, noon B. S. 93. Rx 2 Gm.
5/5/58	Wt. 138, urine 0-0-0. B. S. 112. Rx 1.5 Gm.
6/16/58	Wt. 137, urine 0-0-0. B. S. 93. Rx 1 Gm.
8/11/58	Wt. 138, urine 0-0-0, noon B. S. 132. Rx 0.5 Gm.
10/20/58	Wt. 134, urine 3+ -0-0. B. S. 220. Rx 1 Gm.
11/24/58	Wt. 131½, urine trace -0-0. B. S. 251. Rx 3 Gm.
2/18/59	Wt. 134, urine 0-0-0. B. S. 120. Rx 1 Gm.
4/9/59	Wt. 137½, urine 0-0-0. B. S. normal. Rx 1 Gm.
6/5/59	Wt. 138, urine 0-0-0. B. S. normal. Rx 1 Gm.





Naturetin – reliable therapy in edema and hypertension – maintains a favorable urinary sodium-potassium excretion ratio . . . retains a balanced electrolytic pattern:

"...the increase in urinary output occurs promptly..."[1]

"...the least likely to invoke a negative potassium balance..."[2]

"...a dose of 5 mg. of Naturetin produces a maximal sodium loss."[2]

"...an effective diuretic agent as manifested by the loss in weight..."[3]

"...no apparent influence of clinical importance on the serum electrolytes or white blood count."[3]

"...no untoward reactions were attributed to the drug."[4]

Although Naturetin causes the least serum potassium depletion as compared with other diuretics, supplementary potassium chloride in Naturetin c̄ K provides added protection when treating hypokalemia-prone patients; in conditions where likelihood of electrolyte imbalance is increased or during extended periods of therapy.

Numerous clinical studies confirm the effectiveness[1-15] of Naturetin as a diuretic and antihypertensive – usually in dosages of 5 mg. per day.

■ the most potent diuretic, mg. for mg.—more than 100 times as potent as chlorothiazide ■ prolonged action – in excess of 18 hours ■ maintains its efficacy as a diuretic and antihypertensive even after prolonged or increased dosage use ■ convenient once-a-day dosage – more economical for patients ■ low toxicity – few side effects—low sodium diets not necessary ■ not contraindicated except in complete renal shutdown ■ *in hypertension*—significant lowering of the blood pressure. Naturetin may be used alone or with other antihypertensive drugs in lowered doses.

Supplied: Naturetin Tablets, 5 mg. (scored) and 2.5 mg. Naturetin c̄ K (5 c̄ 500) Tablets (capsule-shaped) containing 5 mg. benzydroflumethiazide and 500 mg. potassium chloride. Naturetin c̄ K (2.5 c̄ 500) Tablets (capsule-shaped) containing 2.5 mg. benzydroflumethiazide and 500 mg. potassium chloride.

SQUIBB

Squibb Quality—the Priceless Ingredient

References: 1. David, N. A.; Porter, G. A., and Gray, R. H.: Monographs on Therapy **5**:60 (Feb.) 1960. 2. Stenberg, E. S., Jr.; Benedetti, A., and Forsham, P. H.: Op. cit. **5**:46 (Feb.) 1960. 3. Fuchs, M.; Moyer, J. H., and Newman, B.E.: Op. cit. **5**:55 (Feb.) 1960. 4. Marriott, H. J. L., and Schamroth, L.: Op. cit. **5**:14 (Feb.) 1960. 5. Ira, G. H., Jr.; Shaw, D. M., and Bogdonoff, M. D.: North Carolina M. J. **21**:19 (Jan.) 1960. 6. Cohen, B. M.: M. Times, to be published. 7. Breneman, G. M., and Keyes, J. W.: Henry Ford Hosp. M. Bull. **7**:281 (Dec.) 1959. 8. Forsham, P. H.: Squibb Clin. Res. Notes **2**:5 (Dec.) 1959. 9. Larson, E.: Op. cit. **2**:10 (Dec.) 1959. 10. Kirkendall, W. M.: Op. cit. **2**:11 (Dec.) 1959. 11. Yu, P. N.: Op. cit. **2**:12 (Dec.) 1959. 12. Weiss, S.; Weiss, J., and Weiss, B.: Op. cit. **2**:13 (Dec.) 1959. 13. Moser, M.: Op. cit. **2**:13 (Dec.) 1959. 14. Kahn, A., and Greenblatt, I. J.: Op. cit. **2**:15 (Dec.) 1959. 15. Grollman, A.: Monographs on Therapy **5**:1 (Feb.) 1960.

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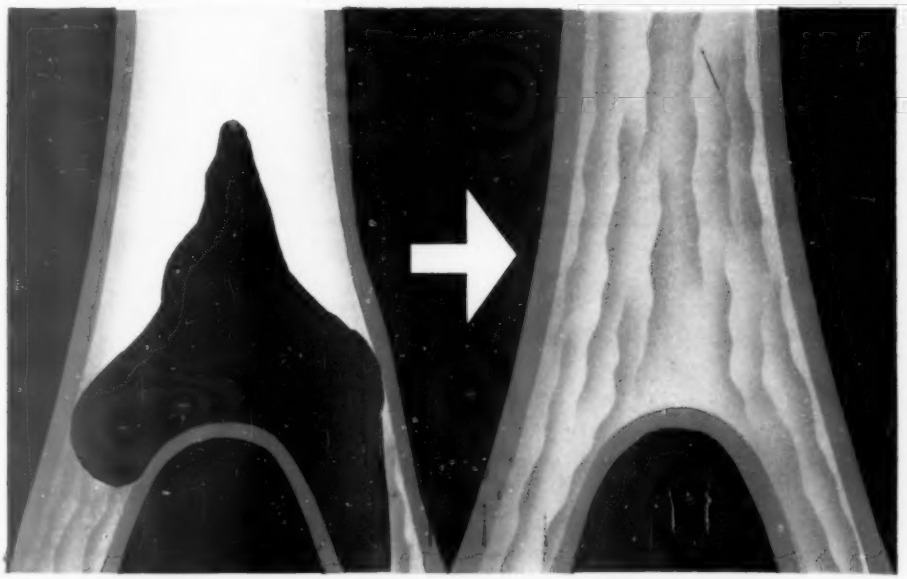

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Why Clinical Judgment Often Dictates Altafur for Peroral, Systemic Therapy of Pyodermas

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ALTAFUR was found "highly satisfactory in most of the primary and secondary bacterial dermatoses treated to date," including "pyodermas . . . caused by antibiotic resistant strains of staphylococci."[1] In a nationwide survey[2] there were **94%** satisfactory results (cured or improved) among 159 patients treated with ALTAFUR for pyodermas.

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99.3% of isolates (214 of 215) from patients with staphylococcal infections—including many antibiotic-resistant strains—proved sensitive in vitro to ALTAFUR in tests conducted across the nation.[3] **99.7%** of staphylococcal isolates (334 of 335) at a large general hospital—including many antibiotic-resistant strains—proved sensitive in vitro to ALTAFUR.[4]

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1. Weiner, A. L.: Paper presented at the Conference on Recent Advances in the Treatment of Chronic Dermatoses, University of Cincinnati (Ohio), Nov. 5, 1959. 2. Compiled by the Medical Department, Eaton Laboratories, from case histories received. 3. Christenson, P. J., and Tracy, C. H.: Current Therapeutic Research 2:22, 1960. 4. Glas, W. W., and Britt, E. M.: Proceedings of the Detroit Symposium on Antibacterial Therapy, Michigan and Wayne County Academies of General Practice, Detroit, Sept. 12, 1959, p. 14. 5. Leming, B. H., Jr.: Ibid., p. 22. 6. Investigators' reports to the Medical Department, Eaton Laboratories.

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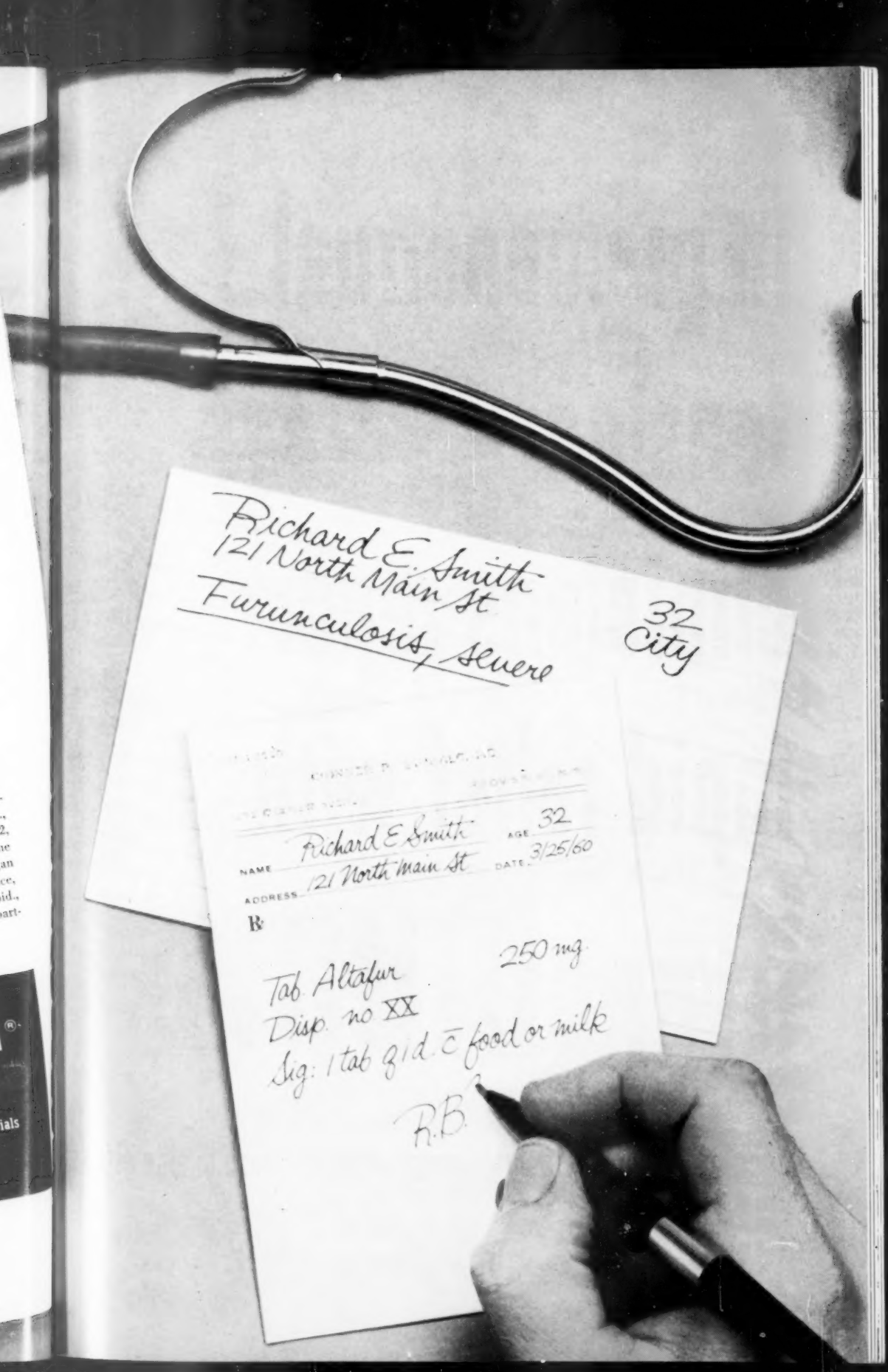
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in premenstrual tension clinicians report rapid relief with

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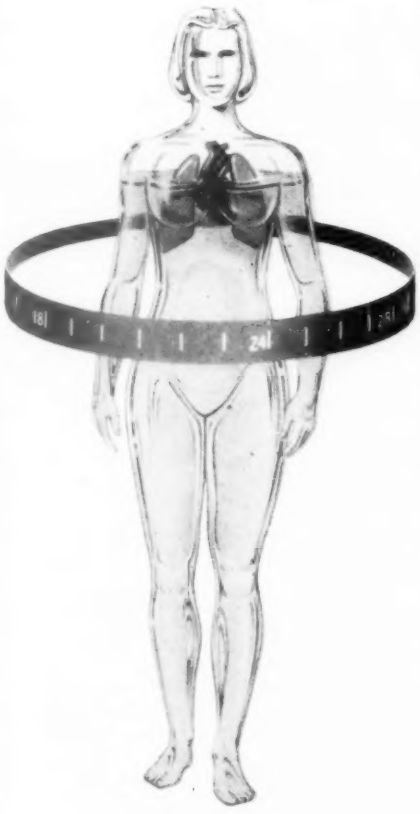
increased potency–without corresponding increase in side effects

Fuchs, M. and Moyer, J.:
Diseases of the Chest 35:314, (March) 1959.

"Premenstrual edema is present in 40% of women and...consists of weight gain, subcutaneous edema, emotional lability, breast turgidity, anxiety and tension." In addition to controlling the objective symptoms of premenstrual tension, HYDRODIURIL may afford relief of subjective complaints including tension, nervousness and headache.



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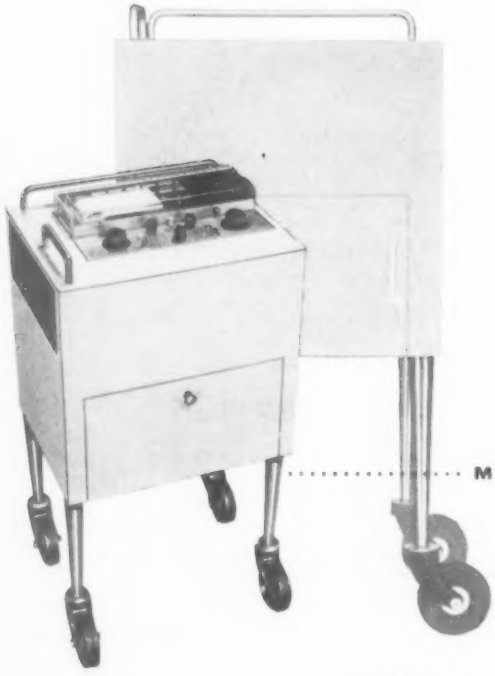

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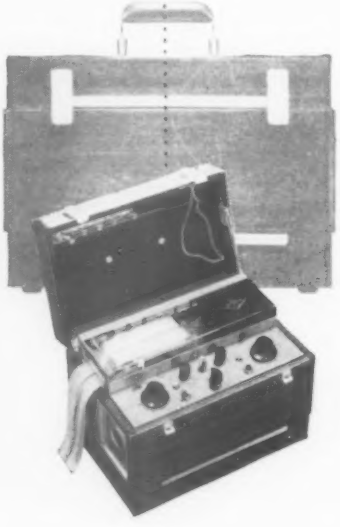

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The Sanborn Model 300 Visette weighs only 18 pounds, is as small as a brief case, has rugged, largely transistorized circuitry. *The Model 100 Viso-Cardiette* is also portable, but expressly designed for use where the versatility of *two* chart speeds, *three* sensitivities, and provision for *monitoring* and *other* types of recording are desired. The third Sanborn instrument is the *Model 100M "Mobile Viso"* — identical in circuitry to the 100, but in a mobile cabinet of either mahogany or rugged, stain-resistant plastic laminate.

Each ECG has *particular* usefulness . . . and each offers *proven* design and performance. Ask your nearby Sanborn man to demonstrate the instrument of your choice — designed for *your* needs.

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References:
1. Yü, T. F., Berns, J. J., and Gutman, A. B.: Arth. & Rheumat. 1:542, 1958. 2. Gutman, A. B., and Yü, T. F.: Bull. N. Y. Acad. Med. 34:367, 1958. 3. Kersley, G. D., Cook, E. R., and Tovey, D. C. J.: Ann. Rheumat. Dis. 17:320, 1958. 4. Gervais, M. A., and Harrison, J.: Ann. Rheumat. Dis. 16:425, 1957.

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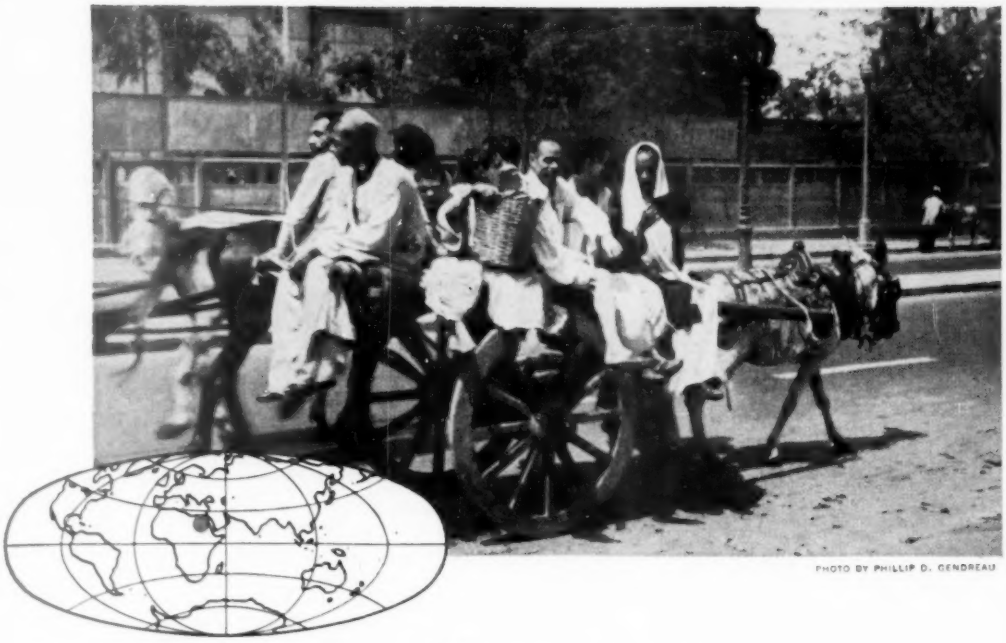

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Musgrave, M. E. and Arm, H. G.: Antibiotic Med. & Clin. Therapy 7:17 (Jan.) 1960.

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References: 1. Dowling, H. F.: Postgrad. Med. *23*:594 (June) 1958. 2. Gimble, A. I.; Shea, J. G., and Katz, S.: Antibiotics Annual 1955-1956 New York, Medical Encyclopedia Inc., 1956, p. 676. 3. Long, P. H., in Kneeland, Y., Jr., and Wortis, S. B.: Bull. New York Acad. Med. *33*:552 (Aug.) 1957. 4. Rein, C. R.; Lewis, L. A., and Dick, L. A.: Antibiotic Med. & Clin. Ther. *4*:771 (Dec.) 1957. 5. Stone, M. L., and Mersheimer, W. L.: Antibiotics Annual 1955-1956, New York, Medical Encyclopedia Inc., 1956, p. 862. 6. Campbell, E. A.; Prigot, A., and Dorsey, G. M.: Antibiotic Med. & Clin. Ther. *4*:817 (Dec.) 1957. 7. Chamberlain, C.; Burros, H. M., and Borromeo, V.: Antibiotic Med. & Clin. Ther. *5*:521 (Aug.) 1958. 8. Fron, P., and Alli, J. H.: Antibiotic Med. & Clin. Ther. *5*:639 (Nov.) 1958.

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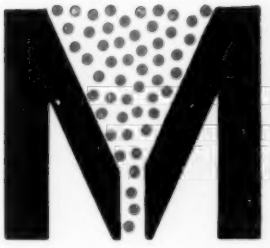

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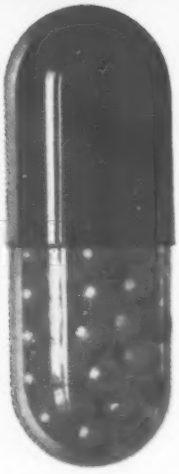

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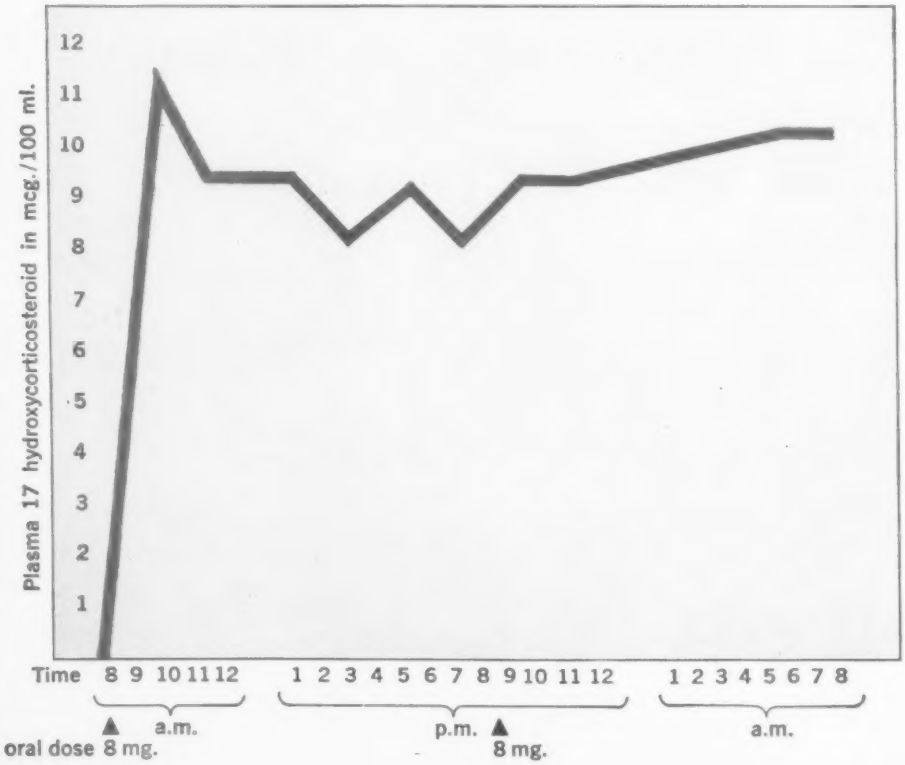

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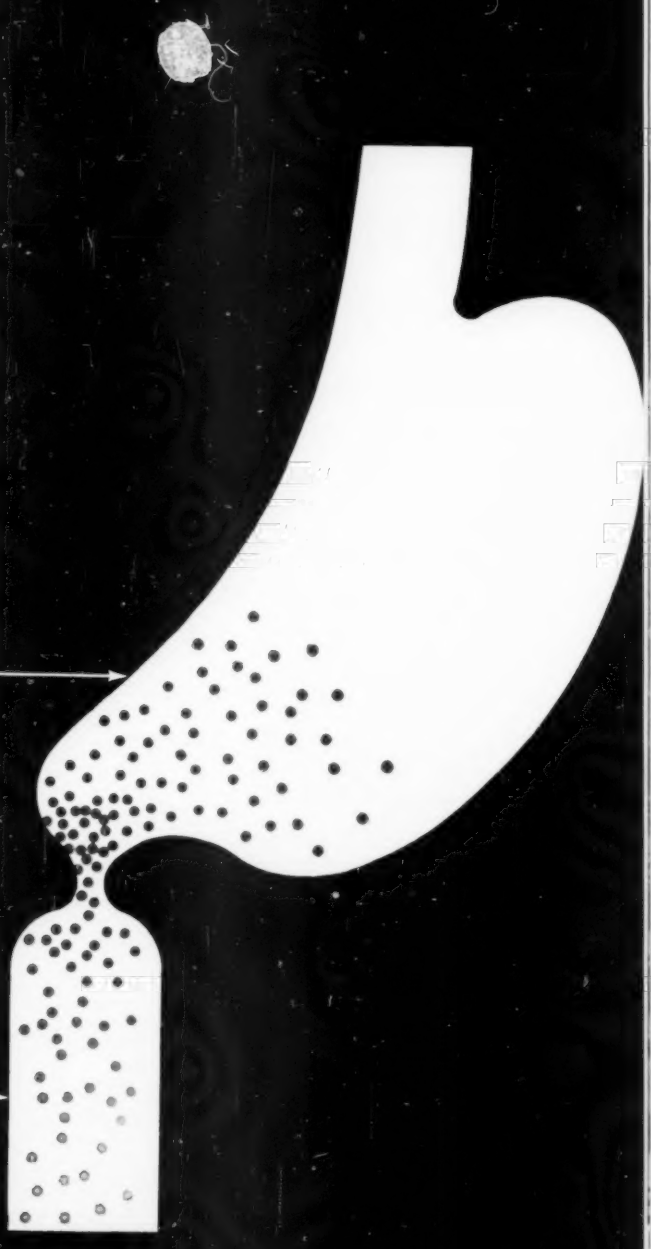

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Bibliography: (1) Paul, O., *et al.*: A.M.A. Arch. Path. *64*:363, 1957. (2) Council on Drugs: J.A.M.A. *172*:240 (Jan. 16) 1960. (3) Pitts, R. F., *et al.*: J. Pharmacol. & Exper. Therap. *123*:89, 1958. (4) Wilkins, R. W.: J.A.M.A. *167*:801 (June 14) 1958. (5) Healey, L. A.; Magid, G. J., and Decker, J. L.: New England J. Med. *261*:1358 (Dec. 31) 1959. (6) Questions and Answers: J.A.M.A. *172*:115 (Jan. 2) 1960. (7) Heineman, H. O.; DeMartini, F. E., and Laragh, J. H.: Am. J. Med. *26*:853, 1959.

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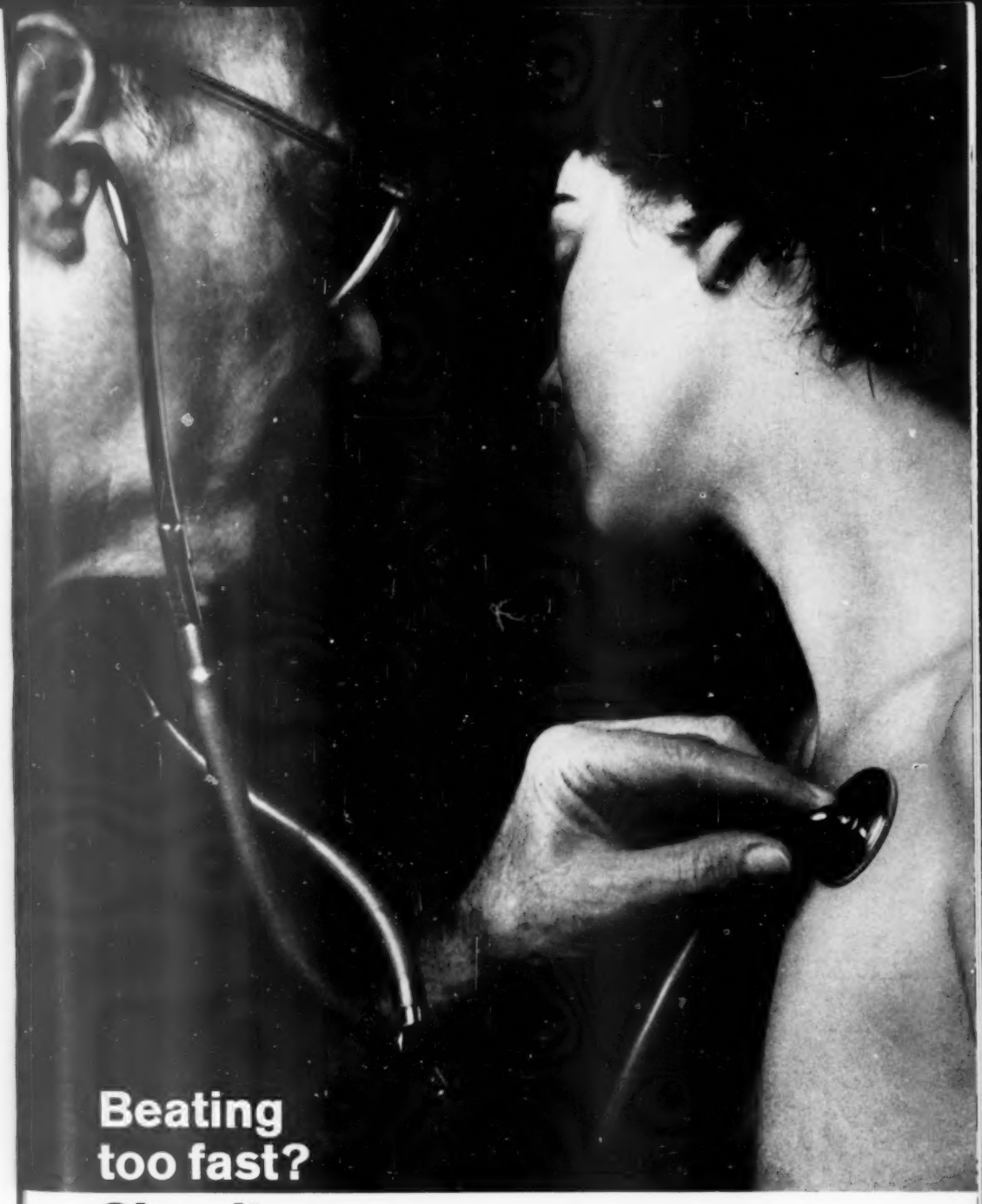

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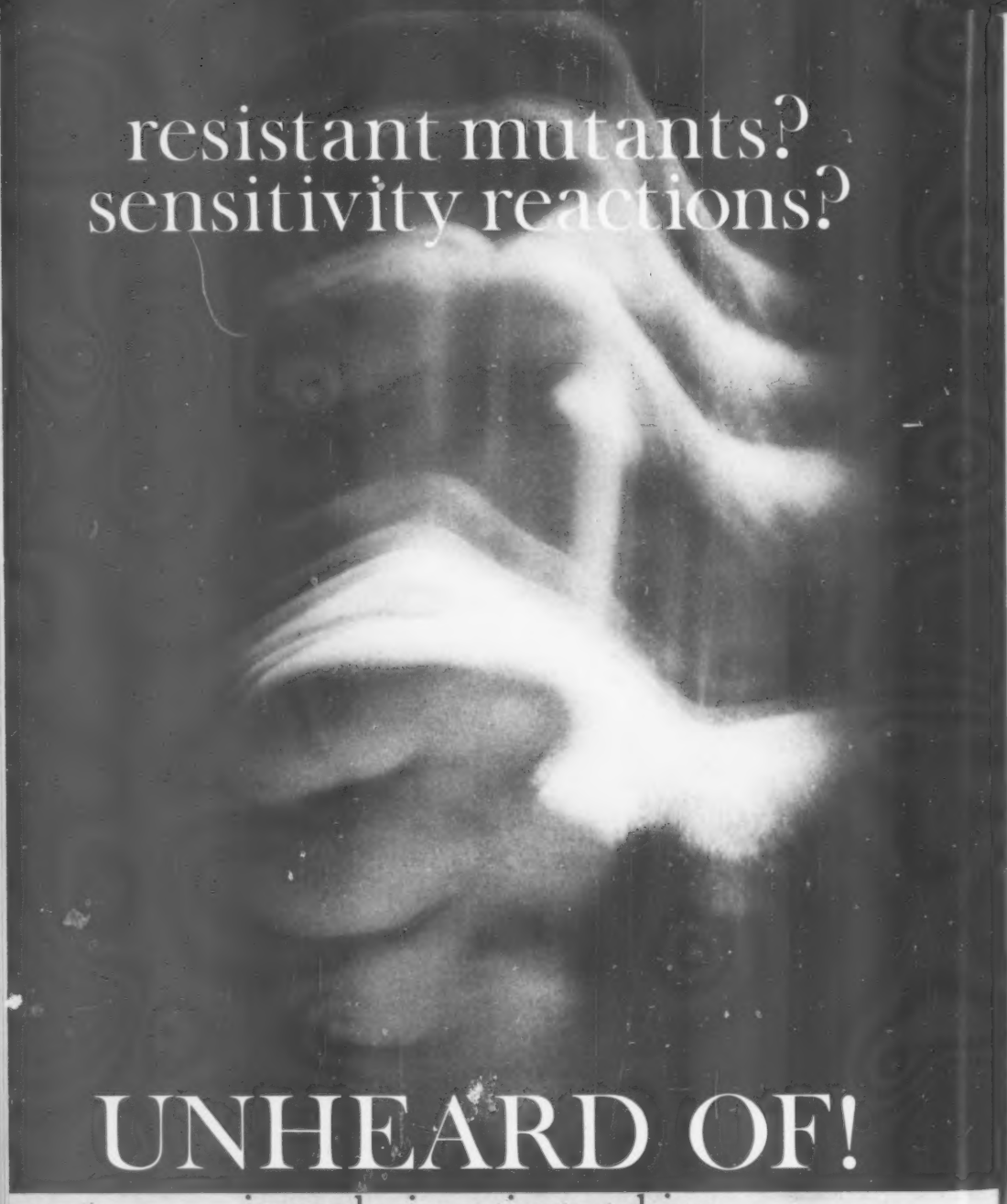

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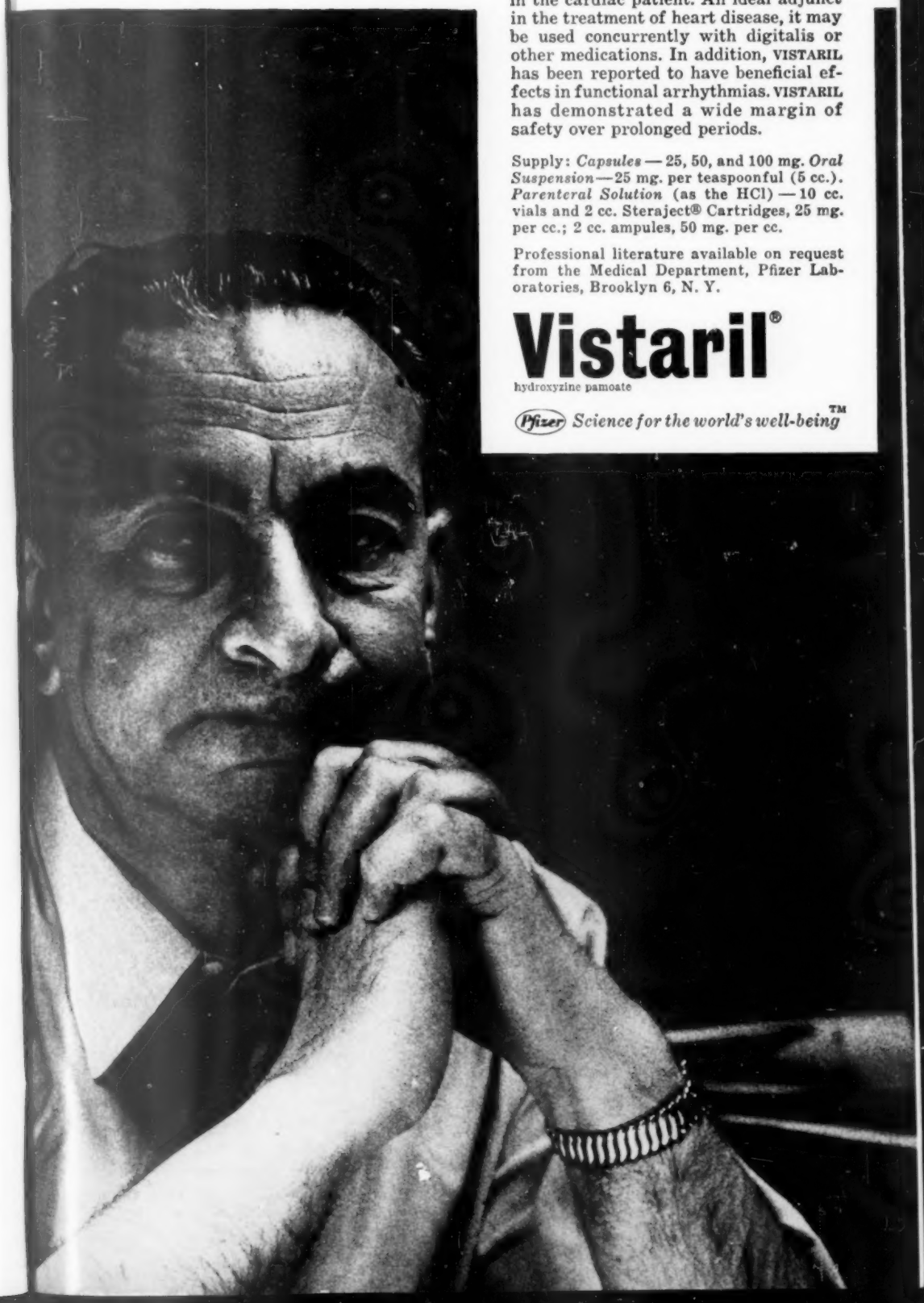

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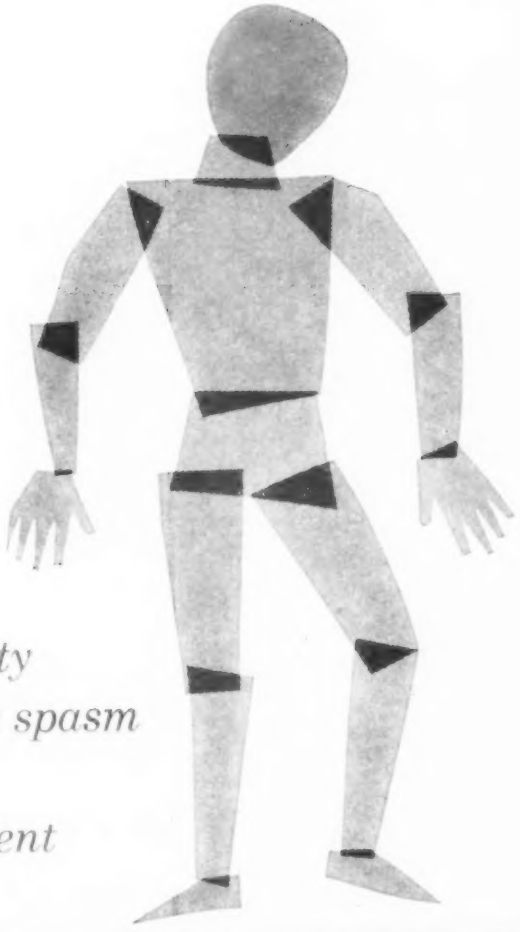

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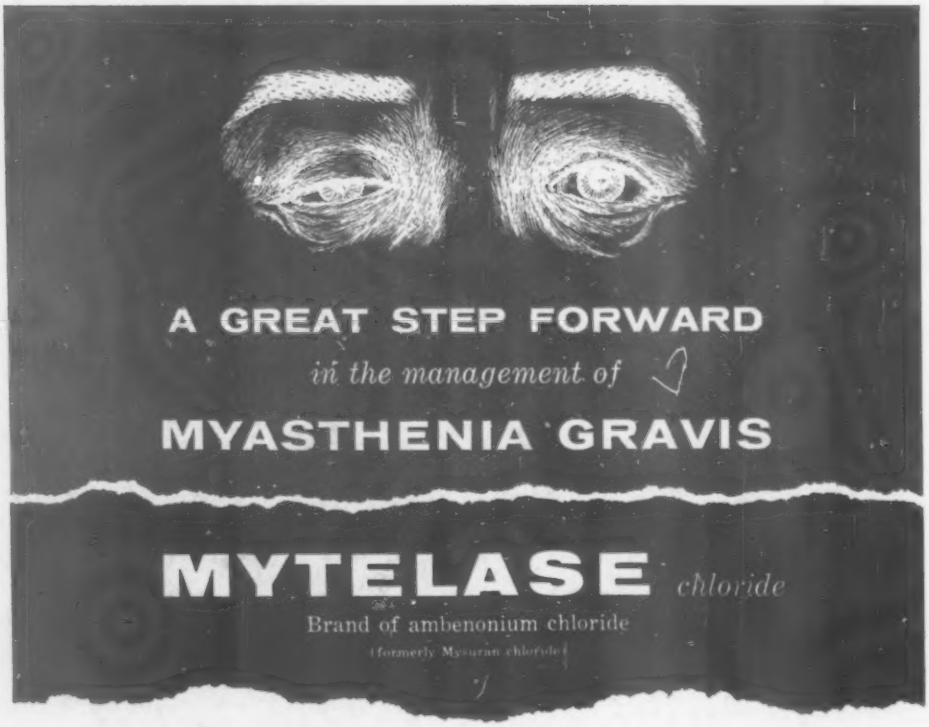

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1. Schwab, R.S.; Marshall, Clare K.; and Timberlake, William: *J.A.M.A.*, 158:625, June 25, 1955.
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